PEARSON

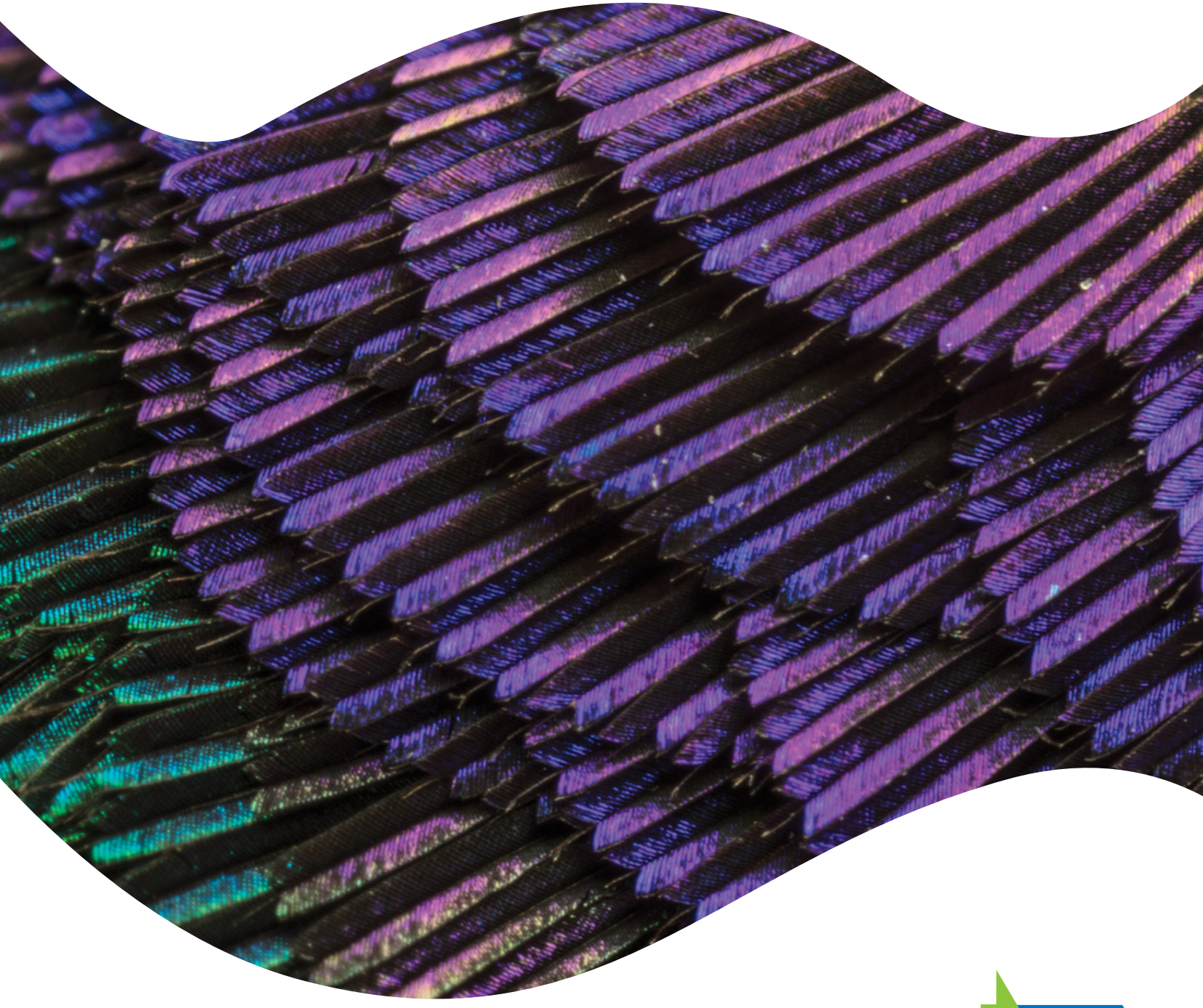
BIOLOGY

QUEENSLAND

STUDENT BOOK

12

UNITS 3 & 4



QCE 2019 SYLLABUS

PEARSON

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STUDENT BOOK

12

UNITS 3 & 4

Pearson

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This syllabus forms part of a new senior assessment and tertiary entrance system in Queensland. Along with other senior syllabuses, it is still being refined in preparation for implementation in schools from 2019.

For the most current syllabus versions and curriculum information please refer to the QCAA website https://www.qcaa.qld.edu.au/

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We are grateful to the following people for their time and expertise in contributing to the Pearson Biology Units 3 and 4 project.

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Kelly Merrin

Contents

ONLINE CHAPTER

Chapter 1 Biology skills and assessment toolkit

Go to your eBook to access this chapter.
Page numbering starts at e1 for this eBook chapter.
Unit 3 starts on page 1, followed by Chapter 2.

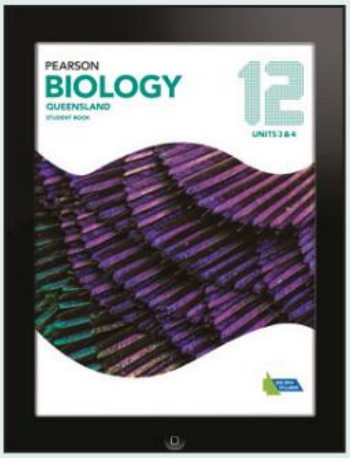
1.1 Successful learning in biology e7

PART A **Working scientifically** **e16**
1.2 Orders of magnitude and estimation e17
1.3 Mathematical basics for biology e22
1.4 Units e25
1.5 Uncertainties in measurement and error e27
1.6 Tables and graphing e35
1.7 Statistics e42

PART B **Student experiment** **e53**
1.8 Research and planning e57
1.9 Conducting an experiment e75
1.10 Results e80
1.11 Communicating and writing a scientific report e87

PART C **Research investigation** **e93**
1.12 Developing the research question from a claim e95
1.13 Finding and choosing suitable resources e99
1.14 Taking and organising notes e107
1.15 Writing a report for the research investigation e112

PART D **Examination** **e116**
1.16 Examination preparation e117



Unit 3: Biodiversity and the interconnectedness of life

Topic 1: Describing biodiversity

CHAPTER 2 **Classification processes** **3**
2.1 Classification 5
2.2 Processes of classification 27
2.3 Classification in practice 46
Mandatory practical 1 56
Chapter review **61**

CHAPTER 3 **Biodiversity** **69**
3.1 Biodiversity and its measurement 70
3.2 Biodiversity and the abiotic environment 91
Mandatory practical 2 120
Chapter review **123**

Topic 2: Ecosystem dynamics

CHAPTER 4 **Ecosystem dynamics** **127**
4.1 Energy transfer 133
4.2 Functioning ecosystems 157
4.3 Population dynamics 176
4.4 Change in ecosystems 191
Mandatory practical 3 218
Chapter review **221**

Unit 3 Review **232**

Unit 4: Heredity and continuity of life

Topic 1: DNA, genes and the continuity of life

CHAPTER 5 **DNA and replication** 247
5.1 DNA structure and genetic code 249
5.2 Genetic expression 263
5.3 Gene regulation 277
5.4 Mutations in genes 286
Chapter review 294

CHAPTER 6 **Cellular replication and variation** 299
6.1 Cellular replication 300
6.2 Mitosis and cytokinesis 308
6.3 Meiosis 312
Chapter review 322

CHAPTER 7 **Inheritance** 325
7.1 Chromosomal abnormality 326
7.2 Genotypes and phenotypes 334
7.3 Monohybrid crosses 343
7.4 Pedigree charts and inheritance patterns 356
7.5 Linked genes, multiple crosses and crossing over 366
Chapter review 374

CHAPTER 8 **Biotechnology** 379
8.1 Biotechnology and genetic engineering 380
8.2 Recombinant DNA, PCR and electrophoresis 394
8.3 DNA sequencing 414
Chapter review 425

Topic 2: Continuity of life on Earth

CHAPTER 9 **Continuity of life on Earth** 431
9.1 Evolution 433
9.2 Natural selection and microevolution 452
9.3 Speciation and macroevolution 475
Mandatory practical 4 498
Chapter review 502

Unit 4 Review 510

GLOSSARY 522
INDEX 532

How to use this book

PEARSON BIOLOGY 12 QUEENSLAND STUDENT BOOK

Pearson Biology 12 Queensland student book has been written to the new QCAA Biology 2019 General Senior Syllabus. The book is an easy-to-use resource that covers Units 3 & 4 and comprehensively addresses the skills and assessment requirements.
Explore how to use this book below.

Design

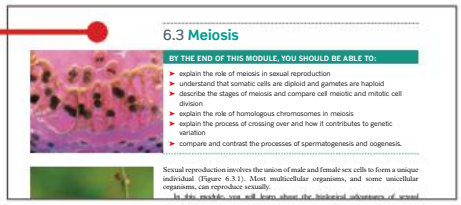
The best-practice literacy and instructional design supports all learners.
A simple to navigate, predictable design enables ease of use. The high-quality, relevant photos and illustrations assist the student understanding of the concepts.

Chapter opener

The syllabus subject matter addressed in each chapter is clearly listed, along with any Science as a human endeavour features and mandatory practicals.

Module opener

Module openers outline the key concepts and skills to be developed and link to the syllabus subject matter listed in the chapter opener.



Science as a human endeavour

The SHE feature provides an opportunity for students to appreciate the development of science and its use and influence on society. The SHE features provide a segue into the development of claims and research questions for the research investigation.

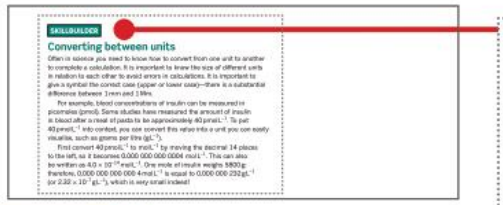
Highlight box

Highlight features focus students' attention on important information such as key definitions, formulas and salient points.

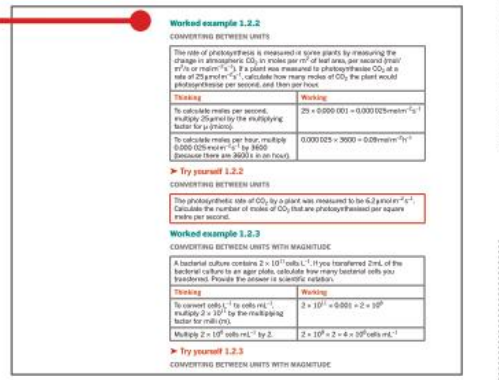
Worked examples

Worked examples use sequential steps of thinking and working to enhance student understanding of subject matter. Each worked example is followed by a try yourself task where students apply their learning to a mirrored problem.

Fully worked solutions to all try yourself problems are available on *Pearson Biology 12 Queensland Teacher Support*.


SKILLBUILDER

Converting between units


Worked example 1.2.2

CONVERTING BETWEEN UNITS

Worked example 1.2.3

CONVERTING BETWEEN UNITS WITH MAGNITUDE

Try yourself 1.2.3

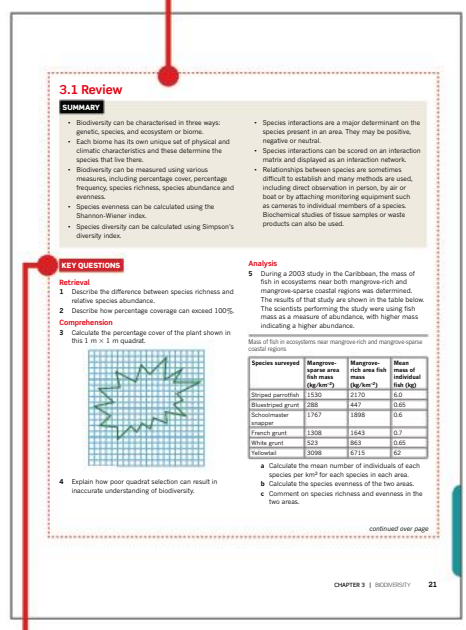
CONVERTING BETWEEN UNITS WITH MAGNITUDE

Skillbuilder

A Skillbuilder outlines a method or technique. Each is instructive and self-contained. Skillbuilders step students through the skill to support science application required when analysing or utilising knowledge.

Module summary

Each module concludes with a summary to help students consolidate the key points and concepts.

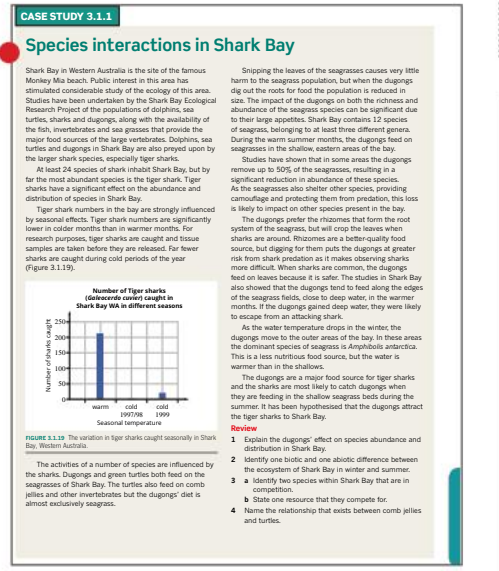

3.1 Review

SUMMARY

KEY QUESTIONS

Case studies

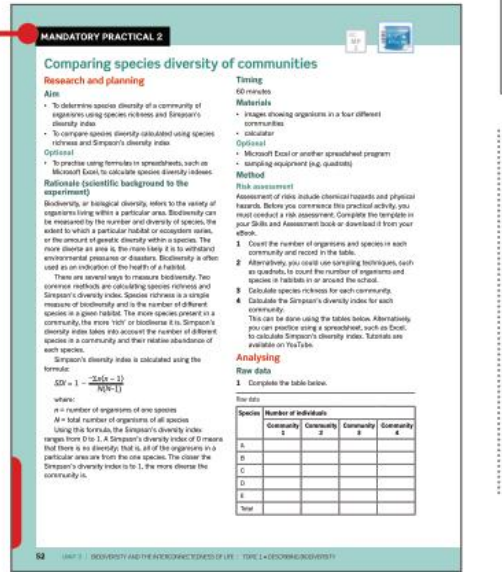
Case studies provide opportunities to engage with current applications and research in biology and address essential syllabus objectives beyond typical learning and understanding conventions. Case studies develop skills in analysing, interpreting, evaluating, decision-making and predicting. Skills are modelled for students in the case studies and then learning is applied in the case study review.


CASE STUDY 3.1.1

Species interactions in Shark Bay

Mandatory practicals

The student book includes all mandatory practicals. Each practical has been trialled and tested to ensure it can be safely performed and yields effective results.


MANDATORY PRACTICAL 2

Comparing species diversity of communities

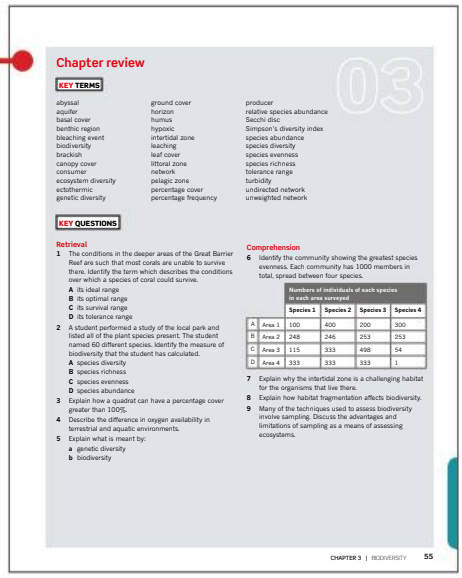
Module review

Key instructions are provided to test students' understanding of concepts of the module. Tasks are carefully categorised under the relevant cognitive level: retrieval, comprehension and analysis, and are developed to assess the syllabus requirements.

How to use this book

Chapter review

Each chapter finishes with a list of key terms covered in the chapter and a set of tasks to test students' abilities to apply the knowledge gained from the chapter.


Chapter review

KEY TERMS

abyssal
aquifer
basal cover
benthic region
bleaching event
biodiversity
brackish
canopy cover
consumer
ecosystem diversity
ectothermic
genetic diversity
ground cover
horizon
humus
hypoxic
intertidal zone
leaching
leaf cover
littoral zone
network
pelagic zone
percentage cover
percentage frequency
producer
relative species abundance
Secchi disc
Simpson's diversity index
species abundance
species diversity
species evenness
species richness
tolerance range
turbidity
undirected network
unweighted network

KEY QUESTIONS

Retrieval

1 The conditions in the deeper areas of the Great Barrier Reef are such that most corals are unable to survive there. Identify the term which describes the conditions over which a species of coral could survive.
A its ideal range
B its optimal range
C its survival range
D its tolerance range

2 A student performed a study of the local park and listed all of the plant species present. The student named 60 different species. Identify the measure of biodiversity that the student has calculated.
A species diversity
B species richness
C species evenness
D species abundance

3 Explain how a quadrat can have a percentage cover greater than 100%.

4 Describe the difference in oxygen availability in terrestrial and aquatic environments.

5 Explain what is meant by:
a genetic diversity
b biodiversity

Comprehension

6 Identify the community showing the greatest species evenness. Each community has 1000 members in total, spread between four species.

	Numbers of individuals of each species in each area surveyed			
	Species 1	Species 2	Species 3	Species 4
A Area 1	100	400	200	300
B Area 2	248	246	253	253
C Area 3	115	333	498	54
D Area 4	333	333	333	1

7 Explain why the intertidal zone is a challenging habitat for the organisms that live there.

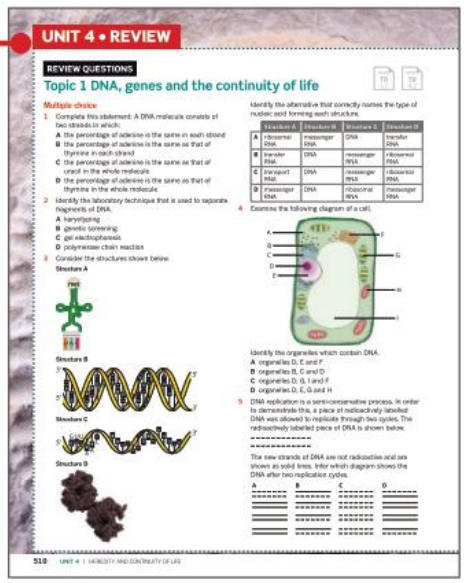
8 Explain how habitat fragmentation affects biodiversity.

9 Many of the techniques used to assess biodiversity involve sampling. Discuss the advantages and limitations of sampling as a means of assessing ecosystems.

CHAPTER 3 | BIODIVERSITY 55

Unit review

Each unit finishes with a comprehensive set of exam-style instructions, including multiple choice and short answer. These review tasks assist students to draw together their knowledge and understanding of the whole unit.


UNIT 4 • REVIEW

REVIEW QUESTIONS

Topic 1 DNA, genes and the continuity of life

Multiple choice

1 Complete this statement: A DNA molecule consists of two strands in which:
A the percentage of adenine is the same in each strand
B the percentage of adenine is the same as that of thymine in each strand
C the percentage of adenine is the same as that of uracil in the whole molecule
D the percentage of adenine is the same as that of thymine in the whole molecule

2 Identify the laboratory technique that is used to separate fragments of DNA.
A karyotyping
B genetic screening
C gel electrophoresis
D polymerase chain reaction

3 Consider the structures shown below.

Structure A
Structure B
Structure C
Structure D

Identify the alternative that correctly names the type of nucleic acid forming each structure.

	Structure A	Structure B	Structure C	Structure D
A	ribosomal RNA	messenger RNA	DNA	transfer RNA
B	transfer RNA	DNA	messenger RNA	ribosomal RNA
C	transport RNA	DNA	messenger RNA	ribosomal RNA
D	messenger RNA	DNA	ribosomal RNA	messenger RNA

4 Examine the following diagram of a cell.

Identify the organelles which contain DNA.
A organelles D, E and F
B organelles B, C and D
C organelles D, G, I and J
D organelles D, E, G and H

5 DNA replication is a semi-conservative process. In order to demonstrate this, a piece of radioactively labelled DNA was allowed to replicate through two cycles. The radioactively labelled piece of DNA is shown below.

The new strands of DNA are not radioactive and are shown as solid lines. Infer which diagram shows the DNA after two replication cycles.

A B C D

510 UNIT 4 | HEREDITY AND CONTINUITY OF LIFE

Glossary

Key terms are shown in **bold** throughout the student book and are listed at the end of each chapter. A comprehensive glossary at the end of the book defines all the key terms. The glossary aligns with the syllabus context and includes the QCAA defined terminology.

Answers

Comprehensive answers and fully worked solutions for all module review tasks, try yourself, Science as a human endeavour, case studies, chapter reviews and unit reviews are provided via the teacher *Pearson Biology 12 Queensland* Reader+ eBook.

Icons

Go To icons make important links to relevant content within the student books in the course. The Go To icons indicate where to engage with Chapter 1 in your eBook.

GO TO ➤

Every mandatory practical is supported by a complementary **SPARKlab** alternative practical.

The ***Pearson Biology Skills and Assessment* book** icons indicate the best time to engage with an activity for practice, application and revision.
The type of activity is indicated as follows:

Worksheet (WS) — WS 3.1.1

Mandatory practical (MP) — MP 4

Practical activity (PA) — PA 3.2.1

Sample assessment task (SAT) — SAT IA1

Topic review (TR) — TR 3.1

The **Reader+** icon indicates when to engage with an asset via your Reader+ eBook. Assets may include videos and interactive activities.

Pearson Biology 12 Queensland

Student book

Pearson Biology 12 Queensland Student Book has been developed by experienced Queensland teachers to address all the requirements of the new QCAA Biology 2019 General Senior Syllabus. The series features the very latest developments and applications of biology, literacy and instructional design to ensure the content and concepts are fully accessible to all students.

Skills and assessment book

The *Pearson Biology 12 Skills and Assessment* book gives students the edge in preparing for all forms of assessment. Specifically prepared to provide opportunities to consolidate, develop and apply subject matter and science inquiry skills, this resource features a toolkit, key knowledge summaries, worksheets, practical activities and guidance, assessment practice and topic review sets.

Reader+ the next generation eBook

Reader+ is our next generation eBook. Students can read, take notes, save bookmarks and more in the one seamless experience. Integrated multimedia (audio/video) and interactive activities enhance and extend the learning experience. In addition, Reader+ provides the digital-only Chapter 1 Biology skills and assessment toolkit.

Teacher support

Pearson Biology 12 Queensland Teacher Support provides:

- complete answers, fully worked solutions or suggested answers to all tasks in the student book, and the skills and assessment book
- expected results, common mistakes, suggested answers and full safety notes and risk assessments for all practical activities
- teaching, learning and assessment programs.

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Access your digital resources at **pearsonplaces.com.au**
Browse and buy at **pearson.com.au**

Understanding the course

Begin your study of the Biology Units 3 & 4 course by clearly establishing what you are required to do and where you can go to for help. Your teacher will teach and guide you through the course. Your Pearson Biology Queensland Unit 3 and 4 resources are a one-stop-shop for support in every aspect of the course. This support is delivered via two resources;

- *Pearson Biology 12 Queensland Student Book*
- *Pearson Biology 12 Queensland Skills and Assessment* book.

The syllabus details all the requirements of the QCE biology course. This syllabus document is long and challenging to interpret. The Pearson resources comprehensively unpack and address all aspects of the syllabus presenting all the subject matter, practical experiences and application opportunities (questions and activities), in a user-friendly way. Refer to the following tables for a summary of where to look for different types of syllabus support, and a summary of where to look for summative assessment support.

Guide to syllabus support in Pearson resources

Where do I go for help with …. ?	***Pearson Biology 12 Queensland Student Book***	***Pearson Biology 12 Queensland Skills and Assessment* book**
ensuring coverage of the syllabus	The student book is organised by units, and the **unit opener** outlines the unit objectives addressed. The student book is further organised into chapters. Each **chapter opener** addresses specific subject matter and mandatory practicals, quoted from the syllabus. **Module openers** within each chapter restate subject matter learning outcomes in student-friendly language.	The skills and assessment book is organised by units, and the **unit opener** outlines the unit objectives addressed. The skills and assessment book is further organised into topics. Each **topic** addresses all of the subject matter and mandatory practicals, quoted from the syllabus.
subject matter and applications	All subject matter is thoroughly covered. **Skillbuilders** and **worked examples** scaffold and model applications. Further opportunities to practice are provided through mirrored **try yourself** problems.	The subject matter is summarised for each topic in **key knowledge** summaries that provide handy notes for revision and study. Extensive opportunities to apply learning from the student book are provided through **worksheets**, **practical activities**, end of **topic review sets** and **sample assessment tasks**.
calculations using algorithms	**Chapter 1 Skills and assessment toolkit** (Reader+ eBook) is a reference tool for refreshing your skills with calculations and other key biology skills. Many **worked examples** in chapters model the use of calculations using algorithms.	Opportunities to apply and practise performing calculations and using algorithms are integrated throughout **worksheets, practical activities** and **question sets.**
mandatory practicals	All **mandatory practicals** are included and have been structured to reflect the structure and assessment objectives of the student experiment to provide consistency and experience.	All **mandatory practicals** are included and have been structured to reflect the structure and assessment objectives of the student experiment to provide consistency and experience. These are more scaffolded than in the student book but provide the same learning outcomes. **Additional practicals** are also included to provide further practice and opportunities for drawing out your student experiment.
general skills in science	**Chapter 1 Skills and assessment toolkit** (Reader+ eBook) is a reference tool covering a wide range of basic skills and understandings, such as use of significant figures, units of measurement, tables and graphs, errors and uncertainties. Additional **Skillbuilders** are included throughout the student book to provide just-in-time teaching moments to step you through developing and applying new skills.	

Where do I go for help with .... ?	*Pearson Biology 12 Queensland Student Book*	*Pearson Biology 12 Queensland Skills and Assessment* book
definitions of key terms	The detailed **glossary** explains all key terms that are in bold throughout the student book. Where the term is defined in the QCAA syllabus, you will find this term and its definition reproduced in the student book glossary.	
practice questions and answers	At the beginning of your student book you will find a **Marzano and Kendall taxonomy guide** which unpacks each of the cognitive verbs and helps you interpret the requirements of the question. This helps ensure that the answer you provide meets the syllabus expectations and maximises the mark you will receive. A large number and variety of questions are available to check for understanding and provide practice. Questions are provided in each: • **module review** • **chapter review** • **unit review.** Questions are organised by complexity of thinking and are presented in instruction form, as per the data test and external assessment. You will also find questions in each **mandatory practical, case study** and **Science as a human endeavour** feature.	**Worksheets** and **practical activities** provide contextualised opportunities to practise and answer questions.
practice exams	**Chapter 1 Skills and assessment toolkit Part D** (Reader+ eBook) **Unit review** questions are presented in a style similar to what you will experience in the exam.	**Topic review** questions are presented in a style similar to what you will experience in the exam.

Guide to summative assessment support in Pearson resources

Where do I go for help with assessments?	*Pearson Biology 12 Queensland Student Book*	*Pearson Biology 12 Queensland Skills and Assessment* book
Data test	**Chapter 1 Skills and assessment toolkit PART A** (Reader+eBook) provides examples and steps for performing calculations, analysing and presenting data.	**Sample assessment task IA1**
Student experiment	**Chapter 1 Skills and assessment toolkit PART B** (Reader+eBook) unpacks the requirements and provides a detailed guide for completing this assessment.	**Sample assessment task IA2**
Research investigation	**Chapter 1 Skills and assessment toolkit PART C** (Reader+eBook) unpacks the requirements and provides a detailed guide for completing this assessment.	**Sample assessment task IA3**
External examination	**Chapter 1 Skills and assessment toolkit PART D** (Reader+eBook) provides helpful tips and guidance in preparing for and showcasing your knowledge in an examination. **Unit review—**exam-style questions	**Topic review**

Understanding assessment instructions

The QCAA Biology 2019 General Senior Syllabus uses an inquiry approach, guiding students in a systematic way to a better understanding of the world.

You will notice as you read through the syllabus for Units 1 to 4 that many terms are underlined. Two examples are provided:

a the General Senior Syllabus objectives

b a sample of topic subject matter.

The syllabus objectives showing the underlining of terms

Syllabus objective		Unit 1	Unit 2	Unit 3	Unit 4
1	describe and explain scientific concepts, theories, models and systems and their limitations	●	●	●	●
2	apply understanding of scientific concepts, theories, models and systems within their limitations	●	●	●	●
3	analyse evidence	●	●	●	●
4	interpret evidence	●	●	●	●
5	investigate phenomena	●	●	●	●
6	evaluate processes, claims and conclusions	●	●	●	●
7	communicate understandings, findings, arguments and conclusions.	●	●	●	●

The QCAA syllabus states that students 'are required to use a range of cognitive processes to demonstrate they meet the syllabus'. Many of the underlined words in the syllabus are action verbs. These verbs are often placed at the start of dot points, to identify the level of thinking (cognitive process) you are expected to demonstrate. Note the action verbs from the two syllabus extracts, summarised in the tables above and at right.

> **Remember the cognitive verbs used in the syllabus subject matter dot points indicate the highest level of thinking and subject engagement to be covered. You will not be assessed at a higher cognitive level.**

A subject matter description from Biology Unit 3, Topic 2 showing the underlining of terms

Subject matter

Changing ecosystems

- explain the concept of ecological succession (refer to pioneer and climax communities and seres)
- differentiate between the two main modes of succession: primary and secondary
- identify the features of pioneer species (ability to fixate nitrogen, tolerance to extreme conditions, rapid germination of seeds, ability to photosynthesise) that make them effective colonisers
- analyse data from the fossil record to observe past ecosystems and changes in biotic and abiotic components
- analyse ecological data to predict temporal and spatial successional changes
- predict the impact of human activity on the reduction of biodiversity and on the magnitude, duration and speed of ecosystem change.
- Mandatory practical: Select and appraise an ecological surveying technique to analyse species diversity between two spatially variant ecosystems of the same classification (e.g. a disturbed and undisturbed dry sclerophyll forest).

UNDERSTANDING COGNITIVE PROCESSES AND VERBS

It is important to understand that the verbs that drive the syllabus objectives and topic subject matter are not randomly chosen. By gaining a better understanding of cognitive verbs, you will be able to respond more satisfactorily to questions and instructions in assessment tasks.

Cognitive verbs are signals to the learner of the type of thinking to be demonstrated. For example:

- the verb *evaluate* indicates that an assessment or judgement must be made
- the verb *describe* requires that an account or outline be provided.

There is a difference between the thinking needed by each of these verbs. To *evaluate* is of a higher level than to *describe*. Generally, the higher the thinking level required in a task, the more challenging it is.

Cognitive verbs can be arranged or classified into different levels of thinking (also known as cognitive processes) ranging from remembering to complex thinking. The QCAA syllabus uses an arrangement (taxonomy) of cognitive processes devised by educational researchers Robert Marzano and John Kendall.

In this arrangement, four levels of cognitive process are identified: retrieval, comprehension, analysis and knowledge utilisation. An outline of these levels is provided in Chart A. A large number of different cognitive verbs are used in the syllabus. These verbs can be aligned with different levels of thinking as shown in Chart B.

Pearson Biology 12 Queensland Student Book provides a comprehensive number of questions and instructions. The review instruction sets are arranged by cognitive levels using the Marzano and Kendall taxonomy and provide students with the opportunity to demonstrate knowledge and application of the subject matter at the following levels:

Module review—retrieval, comprehension and analysis

Chapter review—retrieval, comprehension, analysis and knowledge utilisation

Unit review—retrieval, comprehension, analysis and knowledge utilisation.

CHART A Cognitive processes, as arranged by Marzano and Kendall

Cognitive processes—levels of thinking
(Marzano and Kendall taxonomy)

Retrieval	Comprehension	Analysis	Knowledge utilisation
Level 1—basic level of thinking • Involves remembering, recalling, recognising and executing information.	**Level 2**—higher level of thinking than retrieval • Involves understanding and identifying key information.	**Level 3**—more complex thinking than comprehension • Involves examination of information and the identification of and separation into its separate parts.	**Level 4**—most complex thinking level • Involves applying information to investigate, experiment, problem-solve and make decisions.

Increasing complexity of thinking
Each level of thinking builds upon lower levels. For example, you must be able to retrieve information and comprehend it before you can analyse it.

CHART B Cognitive processes and associated verbs with sample questions and instructions

Cognitive processes, associated verbs and sample instructions and questions

Retrieval	Comprehension	Analysis	Knowledge utilisation
Processes: • recognising • recalling	**Processes:** • executing • integrating • symbolising	**Processes:** • matching • classifying • analysing error • generalising • specifying	**Processes:** • decision-making • problem-solving • experimental inquiry • investigating
Cognitive VERBS	**Cognitive VERBS**	**Cognitive VERBS**	**Cognitive VERBS**
define demonstrate describe identify indicate label list name paraphrase recall recognise (e.g. features) select show state use	calculate (e.g. numerical answer; mathematical processes) clarify comprehend (meaning) construct (e.g. a diagram) demonstrate describe determine develop discuss draw (visual depiction) explain illustrate implement (e.g. plan, proposal) recognise (e.g. features) represent select show sketch summarise symbolise (e.g. through diagram, illustration, model) understand use	analyse apply assess calculate (e.g. numerical answer; mathematical processes) categorise classify compare conclude consider contrast critique deduce derive determine diagnose differentiate discriminate distinguish edit evaluate explore extrapolate identify errors/ problems infer interpret e.g. meaning judge organise/ sequence/ structure predict reflect (on) scrutinise sort	adapt appraise appreciate argue assess comment (make a judgement) conclude conduct (e.g. investigations) construct (e.g. an argument) convince create decide design (e.g. a methodology, an artefact, a proposal) determine develop (e.g. a strategy, product or process) devise discuss/explore draw conclusions evaluate experiment/test (e.g. hypothesis) generate/test (e.g. hypothesis) hypothesise/propose (e.g. argument, concept) investigate/examine (e.g. an argument, statement or conclusion) judge justify/prove (e.g. an argument, statement or conclusion) make decisions manipulate (e.g. language texts; skills; technologies) modify persuade predict (e.g. a result) propose prove realise/resolve (e.g. artistic works) research solve (e.g. problems) synthesise (e.g. information, ideas, components) test

Note that some cognitive verbs appear in more than one cognitive level.

Note that a question may not necessarily include a cognitive verb.

continued over page

CHART B *continued*

Cognitive processes, associated verbs and sample instructions and questions			
Sample instruction and question	**Sample instruction and question**	**Sample instruction and question**	**Sample instruction and question**
Label a phospholipid in the diagram of a cell membrane. What is the structure of a cell membrane according to the fluid mosaic phospholipid bilayer model?	**Explain** the relationship between surface area and the function of gaseous exchange surfaces. What relationship exists between surface area and the function of gaseous exchange surfaces?	**Determine**, using the data provided in the lung capacity schematic, which compartment is the alveoli and which is the capillaries. What can you deduce about gas exchange between alveoli and capillaries, based on the data provided?	**Predict**, using the data provided, which scenario would result in the greatest rate of gas exchange. How will the rate of gas exchange be affected by the change in concentration in the data provided?

UNDERSTANDING THE INSTRUCTION/QUESTION

The cognitive verb alone is not enough to guide understanding of what the question or instruction requires as a response. As seen in Chart B, questions may not include a cognitive verb. In addition, a cognitive verb may apply to more than one level of thinking.

Consider these examples:

Examples	Use of the cognitive verb 'explain'	Scope and context of the instruction or question
	What is the question asking?	**How much do I write?**
Example 1a: Explain diffusion. What is diffusion?	The key aspect/s of the question: **a** definition response —**retrieval** **b** paraphrase response—**comprehension**	A task that focuses on: **a** one key term—diffusion
Example 1b: Explain convection. What is convection?		A task that focuses on one concept: **a** convection
Example 2a: Explain the **difference** between simple and facilitated diffusion. What is **different** between simple and facilitated diffusion?	The key aspect/s of the question: **a** compare and contrast—**analysis**	A task that focuses on: **a** two terms: simple diffusion and facilitated diffusion **b** differences between the two terms
Example 2b: Explain the difference between convection and conduction. What is different between convection and conduction?		A task that focuses on: **a** two terms: convection and conduction **b** differences between the two terms
Example 3a: Determine how you would conduct an experiment using chromatography to **analyse** proteins. What experimental procedure would you use to **analyse** proteins using chromatography?	The key aspect/s of the question: **a** create, design, experiment **b** **knowledge utilisation**	A task that focuses on: **a** two terms: proteins and chromatography **b** their analysis **c** design of an experiment
Example 3b: Determine how you would conduct an experiment using convection to **analyse** the specific heat capacity of a metal. What experimental procedure would you use to analyse the specific heat capacity of a metal using convection?		A task that focuses on: **a** two terms: convection and specific heat capacity **b** their analysis **c** experimental ideas and methods

Strategies for understanding the question or instruction

As you can see, analysing a question or instruction can be quite challenging. This particularly applies when more complex cognitive processes are required.

The following steps provide a framework for understanding and analysing questions and instructions.

Strategies for understanding the question or instruction	**Example** Occasionally a person is born without sweat glands, and cannot secrete water (sweat) onto their skin to lose heat and cool down. Two people are used for an experiment: one with and one without sweat glands. They were placed in warm, dry conditions and data collection included skin and mouth temperatures, and water loss through skin and urine. Which person was born without sweat glands? **Explain** your answer.
1 Underline the cognitive verb/s and identify a plausible thinking level for the verb.	'Which person, was born without sweat glands? Explain your answer.' **Explain** is found in the cognitive verb list for the **comprehension** thinking level.
2 Determine the scope, context of the question and its thinking level.	The scope of the question includes sweat concepts and understanding experimental data for two people under specific conditions. The context is analysing data from an experiment. This targets the **analysis** cognitive level.
3 Consider some cognitive actions from Chart B that are required to complete the question.	The question has depth and complexity. It asks for **analysis** of the data provided. It also requires a **judgement** to be made based on the analysed data.
4 Make sure you know the meaning of every word in the question or instruction.	**Explain** means to 'make an idea or situation plain or clear by describing it in more detail or revealing relevant facts; give an account of; provide additional information' *QCAA definition*.
5 Rephrase the question or instruction in your own words, elaborating on all details required.	Rephrasing might be as another question or instruction. For example: 'Are there differences in the data between the two people that indicate one has sweat glands and one does not? If so, which person has the sweat glands and which doesn't? Then provide plain and clear details using the data to outline why one person was chosen over the other.'

ASSESSMENT TASKS AND COGNITIVE PROCESSES

Pearson Biology 12 Queensland Student Book provides a solid foundation for undertaking all assessment tasks in the syllabus. Comprehensive sets of key instructions and tasks, arranged by cognitive thinking levels, and mirroring the instruction types of the examination, are provided at the end of each module and chapter.

Module reviews have retrieval, comprehension and analysis tasks.

Chapter review tasks cover these three levels as well as knowledge utilisation.

In addition, **unit reviews** provide the opportunity to consolidate and test you on broader areas of subject matter.

Mandatory practicals provide support through practice in responding to cognitive instructions.

This approach will support you in developing the skills and level of application required to complete the assessment tasks.

You are required to complete the following assessment tasks:

a Data test
b Student experiment
c Research investigation
d Examination

The following charts provide an indication of the cognitive processes you should expect to encounter in each type of assessment task. Note that the student experiment and research investigation assessment tasks are designed for thinking at the highest cognitive levels. While retrieval, comprehension and analysis are required to complete the tasks, they are the underlying thinking levels necessary to complete the tasks. In the following charts, the large ticks indicate the focal cognitive thinking level.

Data test (IA1)				
Retrieval	**Comprehension**	**Analysis**	**Knowledge utilisation**	The task requires you to demonstrate thinking that is complex and at the high levels of analysis and knowledge utilisation. Retrieval and comprehension underlie the thinking so data can be analysed in the test.
✔	✔	✔	✔	

Student experiment (IA2)				
Retrieval	**Comprehension**	**Analysis**	**Knowledge utilisation**	The task requires you to demonstrate thinking that is complex and at a high level. Retrieval, comprehension and analysis underlie the experimenting and problem-solving required for this task.
✔	✔	✔	✔	

Research investigation (IA3)				
Retrieval	**Comprehension**	**Analysis**	**Knowledge utilisation**	The task requires you to demonstrate thinking that is complex and at a high level. Retrieval, comprehension and analysis underlie the investigation and decision-making required for this task.
✔	✔	✔	✔	

The examination (EA) will include two papers. Each paper consists of a number of different types of items, including short and combination responses.

	Retrieval	**Comprehension**	**Analysis**	**Knowledge utilisation**	
Short response: • multiple choice • single-word • sentences • calculating using algorithms	✔	✔	✔	✔	Short responses generally draw on factual subject matter in the retrieval and comprehension cognitive processes areas but may require analysis where calculations and data interpretation are involved.
Combination response: • short items requiring single-word, sentence or short paragraph responses • calculating using algorithms • interpreting graphs, tables or diagrams • responding to unseen data and/or stimulus • extended response (300–350 words or equivalent)	✔	✔	✔	✔	The calculations and responses to unseen data move the cognitive processes required to the highest levels of thinking.

DEFINITIONS OF COGNITIVE VERBS

The list that follows provides definitions for cognitive verbs. Where available, the definitions are taken from the QCAA syllabus. Those verbs whose definitions are not in the QCAA syllabus appear in grey text. Refer to the list to clarify exactly what is required when any of these verbs appear in a question or instruction. Verbs are organised according to cognitive levels of thinking.

Increasing complexity of thinking

Level of thinking	Cognitive verb	Definition of cognitive verb
Retrieval: **processes of recognising, recalling, symbolising**	define	give the meaning of a word, phrase, concept or physical quantity; state meaning and identify or describe qualities
	demonstrate	prove or make clear by argument, reasoning or evidence, illustrating with practical example; show by example; give a practical exhibition
	describe	give an account (written or spoken) of a situation, event, pattern or process, or of the characteristics or features of something
	identify	distinguish; locate, recognise and name; establish or indicate who or what someone or something is; provide an answer from a number of possibilities; recognise and state a distinguishing factor or feature
	indicate	suggest, show or recommend a course of action
	label	Identify by applying a name to an object or person
	list	write the names of connected items, usually one below the other
	name	specify or give a label to an object or person
	paraphrase	use different words to convey the same meaning
	recall	remember; present remembered ideas, facts or experiences; bring something back into thought, attention or into one's mind
	recognise	identify or recall particular features of information from knowledge; identify that an item, characteristic or quality exists; perceive as existing or true; be aware of or acknowledge
	select	choose in preference to another or others; pick out
	show	provide the relevant reasoning to support a response
	state	express something definitely and clearly
	use	operate or put into effect; apply knowledge or rules to put theory into practice

Increasing complexity of thinking

Level of thinking	Cognitive verb	Definition of cognitive verb
Comprehension: **processes of integrating, symbolising**	calculate (e.g. numerical answer, mathematical processes)	work out using mathematical processes and determine by reasoning
	clarify	make clear or intelligible
	comprehend (meaning)	understand the meaning or nature of; grasp mentally
	construct	create or put together (e.g. an argument) by arranging ideas or items; display information in a diagrammatic or logical form: make; build
	demonstrate	prove or make clear by argument, reasoning or evidence, illustrating with practical example; show by example; give a practical exhibition
	describe	give an account (written or spoken) of a situation, event, pattern or process, or of the characteristics or features of something
	determine	establish, conclude or ascertain after consideration, observation or calculation; decide or come to a resolution
	develop	elaborate, expand or enlarge in detail; add detail and fullness to; cause to become more complex or intricate
	discuss	examine by argument; sift the considerations for and against; debate; talk or write about a topic, including a range of arguments, factors or hypotheses; consider, taking into account different issues and ideas, points for and/or against, and supporting opinions or conclusions with evidence
	draw (visual depiction)	produce a picture, diagram or other visual representation
	explain	make an idea or situation plain or clear by describing it in more detail or revealing relevant facts; give an account; provide additional information
	illustrate	provide pictures, provide an example for a point being made
	implement	put something into effect, e.g. a plan or proposal
	recognise	identify or recall particular features from knowledge; identify that an item, characteristic or quality exists; perceive as existing or true; be aware of or acknowledge
	represent	scientific representations are a verbal, physical or mathematical demonstration of understanding of a science concept or concepts; a concept can be represented in a range of ways and using multiple models (ACARA 2015c)
	show	provide the relevant reasoning to support a response
	sketch	execute a drawing or painting in simple form, giving essential features but not necessarily with detail or accuracy
	summarise	give a brief statement of a general theme or major point/s; present ideas and information in fewer words and in sequence
	symbolise	represent or identify by a symbol or symbols
	understand	perceive what is meant by something; grasp; be familiar with (e.g. an idea); construct meaning from messages, including oral, written and graphic communication
	use (models)	operate or put into effect; apply knowledge or rules to put theory into practice

Increasing complexity of thinking

Level of thinking	Cognitive verb	Definition of cognitive verb
Analysis: **processes of matching, classifying, analysing errors, generalising, specifying**	analyse	dissect to ascertain and examine constituent parts and/or their relationships; break down or examine in order to identify the essential elements, features, components or structure; determine the logic and reasonableness of information; examine or consider something in order to explain and interpret it, for the purpose of finding meaning or relationships and identifying patterns, similarities and differences
	apply	use knowledge and understanding in response to a given situation or circumstance; carry out or use a procedure in a given or particular situation
	assess	measure, determine, evaluate, estimate or make a judgment about the value, quality, outcomes, results, size, significance, nature or extent of something
	calculate	work out using mathematical processes and determine by reasoning.
	categorise	place in or assign to a particular class or group; arrange or order by classes or categories; classify, sort out, sort, separate
	classify	arrange, distribute or order in classes or categories according to shared qualities or characteristics
	compare	display recognition of similarities and differences and recognise the significance of these similarities and differences
	conclude	judgment based on evidence (ACARA 2015c)
	conjecture	infer from what is known; extend the application of something (e.g. a method or conclusion) to an unknown situation by assuming that existing trends will continue or similar methods will be applicable
	contrast	display recognition of differences by deliberate juxtaposition of contrary elements; show how things are different or opposite; give an account of the differences between two or more items or situations, referring to both or all of them throughout
	critique	review (e.g. a theory, practice, performance) in a detailed, analytical and critical way
	deduce	reach a conclusion that is necessarily true, provided a given set of assumptions is true; arrive at, reach or draw a logical conclusion from reasoning and the information given
	determine	establish, conclude or ascertain after consideration, observation, investigation or calculation; decide or come to a resolution
	diagnose	identify the nature of a problem or illness
	differentiate	identify the difference/s in or between two or more things; distinguish, discriminate; recognise or ascertain what makes something distinct from similar things; in mathematics, obtain the derivative of a function
	discriminate	note, observe or recognise a difference; make or constitute a distinction in or between; differentiate; note or distinguish as different
	distinguish	recognise as distinct or different; note points of difference between; discriminate; discern; make clear a difference/s between two or more concepts or items
	edit	correct written material by careful checking
	evaluate	make an appraisal by weighing up or assessing strengths, implications and limitations; make judgments about ideas, works, solutions or methods in relation to selected criteria; examine and determine the merit, value or significance of something, based on criteria
	explore	look into both closely and broadly; scrutinise; inquire into or discuss something in detail
	extrapolate	infer or estimate by extending or projecting known information
	identify (categories, errors, problems)	recognise and establish things such as groupings of similar items, mistakes or issues
	infer	derive or conclude something from evidence and reasoning, rather than from explicit statements; listen or read beyond what has been literally expressed; imply or hint at

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continued

Increasing complexity of thinking

Level of thinking	Cognitive verb	Definition of cognitive verb
Analysis: **processes of matching, classifying, analysing errors, generalising, specifying** *(continued)*	interpret	use knowledge and understanding to recognise trends and draw conclusions from given information; make clear or explicit; elucidate or understand in a particular way; bring out the meaning of, e.g. a dramatic or musical work, by performance or execution; bring out the meaning of an artwork by artistic representation or performance; give one's own interpretation of; identify or draw meaning from, or give meaning to, information presented in various forms, such as words, symbols, pictures or graphs
	judge	form an opinion or conclusion about; apply both procedural and deliberative operations to make a determination
	organise	arrange, order; form as or into a whole consisting of interdependent or coordinated parts, especially for harmonious or united action
	predict	give an expected result of an upcoming action or event; suggest what may happen based on available information
	reflect on	think about deeply and carefully
	scrutinise	to examine closely or critically (Macquarie 2015)
	sort	arrange in prescribed groupings or order
Knowledge utilisation: **processes of investigating, experimenting, decision-making, problem-solving**	adapt	modify or change something for a new purpose or use
	appraise	valuate the worth, significance or status of something; judge or consider a text or piece of work
	appreciate	recognise or make a judgment about the value or worth of something; understand fully; grasp the full implications of
	argue	give reasons for or against something; challenge or debate an issue or idea; persuade, prove or try to prove by giving reasons
	assess	measure, determine, evaluate, estimate or make a judgment about the value, quality, outcomes, results, size, significance, nature or extent of something
	comment	express an opinion, observation or reaction in speech or writing; give a judgment based on a given statement or result of a calculation
	conduct	direct an action or course; manage; organise; carry out
	conclude (conclusion)	a judgment based on evidence (ACARA 2015c)
	construct	create or pull together (e.g. an argument) by arranging ideas or items; display information in a diagrammatic or logical form; make; build
	convince (convincing)	persuade by argument or proof; leaving no margin of doubt; clear; capable of causing someone to believe that something is true or real; persuading or assuring by argument or evidence; appearing worthy of belief; credible or plausible
	create	bring something into being or existence; produce or evolve from one's own thought or imagination; reorganise or put elements together into a new pattern or structure or to form a coherent or functional whole
	design	produce a plan, simulation, model or similar; plan, form or conceive in the mind; in English, select, organise and use particular elements in the process of text construction for particular purposes; these elements may be linguistic (words), visual (images), audio (sounds), gestural (body language), spatial (arrangement on the page or screen) and multimodal (a combination of more than one)
	decide	reach a resolution as a result of consideration; make a choice from a number of alternatives
	determine	establish, conclude or ascertain after consideration, observation, investigation or calculation; decide or come to a resolution
	develop	elaborate, expand or enlarge in detail; add detail and fullness to; cause to become more complex or intricate
	devise	think out; plan; contrive; invent

continued

Level of thinking	Cognitive verb	Definition of cognitive verb
Knowledge utilisation: **processes of investigating, experimenting, decision-making, problem-solving** *(continued)*	discuss	examine by argument; sift the considerations for and against; debate; talk or write about a topic, including a range of arguments, factors or hypotheses; consider, taking into account different issues and ideas, points for and/or against, and supporting opinions or conclusions with evidence
	draw conclusions (conclusion)	a judgment based on evidence (ACARA 2015c)
	evaluate	make an appraisal by weighing up or assessing strengths, implications and limitations; make judgments about ideas, works, solutions or methods in relation to selected criteria; examine and determine the merit, value or significance of something, based on criteria
	experiment	try out or test new ideas or methods, especially in order to discover or prove something; undertake or perform a scientific procedure to test a hypothesis, make a discovery or demonstrate a known fact
	explore	inquire into something or discuss in detail
	generate	produce; create; bring into existence
	hypothesise	formulate a supposition to account for known facts or observed occurrences; conjecture, theorise, speculate; especially on uncertain or tentative grounds
	investigate	carry out an examination or formal inquiry in order to establish or obtain facts and reach new conclusions; search, inquire into, interpret and draw conclusions about data and information
	judge	form an opinion or conclusion about; apply both procedural and deliberative operations to make a determination
	justify	give reasons or evidence to support an answer, response or conclusion; show or prove how an argument, statement or conclusion is right or reasonable
	make decisions	select from available options; weigh up positives and negatives of each option and consider all the alternatives to arrive at a position
	manipulate	adapt or change to suit one's purpose
	modify	change the form or qualities of; make partial or minor changes to something
	persuade (persuasive)	capable of changing someone's ideas, opinions or beliefs; appearing worthy of approval or acceptance; (of an argument or statement) communicating reasonably or credibly
	predict	give an expected result of an upcoming action or event; suggest what may happen based on available information
	propose	put forward (e.g. a point of view, idea, argument, suggestion) for consideration or action
	prove	use a sequence of steps to obtain the required result in a formal way
	research	to locate, gather, record, attribute and analyse information in order to develop understanding (ACARA 2015c)
	resolve	in the Arts, consolidate and communicate intent through a synthesis of ideas and application of media to express meaning
	solve	find an answer to, explanation for, or means of dealing with (e.g. a problem); work out the answer or solution to (e.g. a mathematical problem); obtain the answer/s using algebraic, numerical and/or graphical methods
	synthesise	combine different parts or elements
	test	take measures to check the quality, performance or reliability of something

Increasing complexity of thinking

CHAPTER

01 Biology skills and assessment toolkit

This chapter provides important information and support in the study of the Queensland Curriculum & Assessment Authority (QCAA) Biology syllabus for Units 3 and 4.

The Biology skills and assessment toolkit is designed to be used as a reference tool. It should be consulted on a need-to-know basis, where relevant, during this course of study. It is not intended that this chapter be worked through as a whole.

Focus

The chapter focuses on providing support and guidance in:

- successful learning in biology
  - representations in biology
  - developing strategies for learning
  - reducing barriers to learning
- developing and applying scientific skills
  - mathematical and statistical processes used in biology
  - SI units
  - visual representations
  - graphical representations
  - measurement, errors and uncertainty
- responding to the assessment tasks
  - data test (ISMG: IA1)
    - understanding, analysing and interpreting data and statistics
  - student experiment (ISMG: IA2)
    - developing a research question or hypothesis
    - considering variables, risks and types of data
    - planning methodology
    - presenting, analysing and interpreting data
    - writing the scientific report
  - research investigation (ISMG: IA3)
    - understanding and analysing claims
    - developing research questions
    - evaluating resources
    - taking notes
    - writing a scientific report
  - examination (EA)
    - understanding the features and conditions of the two exam papers
    - strategies to apply through the course to enhance learning
    - strategies for revising and consolidating learning
    - techniques and hints for sitting the exam.

Chapter organisation

The chapter is organised in four parts:

- Part A: Working scientifically
- Part B: Student experiment
- Part C: Research investigation
- Part D: Examination.

An outline of these parts is provided on the following pages.

Please note: The examples provided in this chapter assist in modelling how students may arrive at a testable research question or hypothesis. All summative assessment tasks will need to be officially endorsed prior to undertaking and should be checked to ensure they address the QCAA instrument-specific marking guide criteria. Experiments must also be carefully reviewed in consultation with your science teacher or school laboratory technician.

QCAA Biology syllabus objectives

- describe and explain scientific concepts, theories, models and systems and their limitations
- apply understanding of scientific concepts, theories, models and systems within their limitations
- analyse evidence
- interpret evidence
- investigate phenomena
- evaluate processes, claims and conclusions
- communicate understandings, findings, arguments and conclusions.

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Part A: Working scientifically

Part A focuses on basic mathematical skills and their applications. It features many worked examples and gives you opportunities to try them yourself. These skills are developed throughout the course, and are assessed, directly and indirectly, in assessment tasks and drawn on when you analyse data in practical investigations.

Engaging with Part A will help you develop the skills you will use in the data test and when you are undertaking a range of mandatory and suggested practicals. You will also find these skills useful when you complete sections of the student experiment, research investigation and examination.

Refer to the following outline of *Part A: Working scientifically* to learn, revise and practise skills in areas where you need help.

Module	Look here for:	eBook page
1.2 Orders of magnitude and estimation	• scientific notation • transforming decimal notations to scientific notation • orders of magnitude • converting between units	e17
1.3 Mathematical basics for biology	• examples of maths used in biology	e22
1.4 Units	• measurement and units • correct use of unit symbols	e25
1.5 Uncertainties in measurement and error	• explanations of the terms uncertainty, error, accuracy and precision • causes of errors • calculations with uncertainty and error • relative uncertainty • writing measurements with uncertainty and error values • writing measurement and calculations to the correct significant figure	e27
1.6 Tables and graphing	• arranging and recording data in tables • converting data from tables to graphs • types of graphs (scatterplots, line graphs, bar and column graphs, pie charts) • outliers • representing missing data on graphs	e35
1.7 Statistics	• range • measures of central tendency • standard deviation • standard error • confidence interval • student *t*-test • *P* value	e42

GO TO ➤ your R+ eBook to access Chapter 1: Biology Skills and Assessment Toolkit.

Part B: Student experiment (IA2)

Part B focuses on the student experiment. This internal assessment requires students to follow the full scientific method over an extended and defined period of time. You need to develop your own research question to investigate, based on an initial practical already completed in class.

Part B supports you through all aspects of the student experiment and explains the QCAA objectives and instrument-specific marking guide (ISMG IA2) for the assessment.

Part B has examples of how to modify (i.e. refine, extend or redirect) the class practical and write a research question or hypothesis. Use the step-by-step guide to evaluate the quality of your question or hypothesis. Look closely at particular sections of Part B, when needed, to reinforce your knowledge and understanding of scientific methodology.

Refer to Part B for support on data types, data collection and the analysis of data to draw valid conclusions, including how to identify uncertainty and limitations through errors in data, reliability and relationships between data as well as how to determine validity. Support material on scientific writing style and the structure of the report will help you to write up your scientific report.

Refer to the following outline of *Part B: Student experiment* to learn, revise and practise skills in areas where you need help.

Module	Look here for:	eBook page
1.8 Research and planning	• types of variables • modifying, refining, extending or redirecting the initial practical • developing a research question or hypothesis • evaluating the research question or hypothesis • using a scientific journal to keep a record • validity and reliability • quantitative and qualitative data • nominal, ordinal, discrete and continuous data • risk assessments • chemical codes and symbols	e57
1.9 Conducting an experiment	• determining what data is relevant • determining what data is sufficient • collecting data • considerations for planning an experiment • precision of scientific instruments	e75
1.10 Results	• analysing raw data • identifying errors (mistakes, systematic errors, random errors) • analysing precision • analysing validity and theoretical relationships • interpreting results	e80
1.11 Communicating and writing a scientific report	• sections of a report • scientific writing style • writing a scientific report • acknowledging sources • addressing the ISMG	e87

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Part C: Research investigation (IA3)

Part C focuses on the research investigation. This internal assessment requires students to gather secondary evidence on a research question over an extended and defined period of time. Students develop their own research question to investigate, based on a claim (provided by your teacher) related to the course.

Part C supports you through all aspects of the research investigation and explains the QCAA objectives and instrument-specific marking guide (ISMG IA3) for the assessment.

Use Part C for help with writing your research question. The examples and analysis of claims will help you to develop your own research question. Be guided by the information about locating and evaluating suitable secondary sources for your research, including how to identify errors in data, validity and relationships between data.

Part C also has support material on the scientific writing style and the structure of scientific reports to help you to write your scientific report. It provides a brief overview of different ways to present the report and gives details on the literature review format.

Refer to the following outline of *Part C: Research investigation* to learn, revise and practise skills in areas where you need help.

Module	Look here for:	eBook page
1.12 Developing the research question from a claim	• analysing a claim • identifying variables and measurable terms in the claim • examples of claims and questions developed from them • guidelines for developing a research question • developing a research question • refining a research question	e95
1.13 Finding and choosing suitable resources	• understanding the difference between primary and secondary sources • locating resources • determining the reliability and validity of resources	e99
1.14 Taking and organising notes	• recording notes in a scientific journal • paraphrasing information • different ways to record information • recording data and results • recording information about sources	e107
1.15 Writing a report for the research investigation	• different ways to present the report • presenting the report as a literature review • features of a literature review • structure of a literature review	e112

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Part D: Examination

Part D focuses on the summative external assessment (EA). The EA requires students to complete two papers. Each paper covers the whole biology course: Unit 3 and Unit 4. The examination constitutes 50% of the total assessment for the course.

Part D supports you through all aspects of the EA examination process. Refer to this section early in the year, not just in the weeks before the examination. Effective exam preparation needs ongoing consolidation of learning and revision of course material at regular intervals. Effective preparation begins with a sound understanding of the course and its requirements. It is also important that you know where you can go for support and what sort of support is available to you through the course.

The examination requires that you use skills and strategies that are not necessarily used in the student experiment and research investigation. The data test and examination are similar in the strategies you should apply to complete them. There is possibly more anxiety felt by students regarding the examination, as it accounts for 50% of the total mark in biology.

Part D guides you through all aspects of preparation for the examination. Refer to it to get tips on strategies to help you consolidate your learning and for revision hints and suggestions. Before completing practice exams, read through the list of strategies to use during perusal (reading) and writing time. Apply these as you answer each practice paper. Sitting the exam is more than having subject matter understanding and skills. It is also about exam technique, as this can boost your performance. Refer to Part D as needed throughout the year.

Refer to the following outline of *Part D: Examination* to learn about strategies and techniques to enhance your learning, revision and examination performance.

Module	Look here for:	eBook page
1.16 Examination preparation	• overview of the two papers (duration, content, response items and equipment) • strategies for consolidating learning • strategies for revising and practising • strategies for sitting the exam	e117

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1.1 Successful learning in biology

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- appreciate effective strategies for high-impact learning
- develop your understanding of the role of representations
- develop connections and provide strategies for learning
- appreciate how a knowledge of the four representations in biology can enhance your learning
- develop a deeper understanding of concepts and applications in biology
- identify barriers to your learning
- identify strategies to reduce your barriers to learning.

HIGH-IMPACT LEARNING STRATEGIES

Recent research into best practices for teaching and learning to optimise student learning has highlighted some interesting findings. Many of the results relate to teachers and their classroom strategies. There are, however, some important learning strategies that you can apply that will make learning more effective and assist you in working through the challenges of learning. The high-impact learning strategies discussed in the chart below are proven ways to improve learning outcomes. These strategies are not exclusive to learning biology. They can be applied to all learning in all subjects and situations. At the core of all learning is the idea that you, the learner, gain increasing regulation and control of your own learning, propelling you along the path of lifelong learning.

Table 1.1.1 identifies three key characteristics—attitudes, behaviours and metacognition—and associated strategies that positively influence learning outcomes. Research has shown that the listed strategies are among the most highly effective, and through applying these, students directly influence their learning and achievement.

TABLE 1.1.1 High-impact student learning characteristics and strategies

	Attitudes	Behaviours	Metacognition
Characteristics	Attitude relates to your frame of mind, your approach to your learning and your responses to challenges to produce your desired level of learning.	Behaviour relates to the actions you take and habits you develop and implement as learning strategies.	Metacognition relates to thinking about how you think, and gaining of awareness of how you learn. Metacognition is very complex thinking and involves you reflecting on how you best learn.
Strategies	• Effort: Increasing the level of effort can improve your abilities in and mastery of a subject. Effort can be directed at subject matter and skill competence. • Motivation: The desire to learn for learnings' sake rather than learning specifically. If you are sometimes lacking in motivation, try breaking tasks down into smaller chunks and reward yourself when each task is finished.	• Practise: Practise, practise and more practise. Few people have such natural ability that they are experts. Most people devote hours repeating and retrying tasks in order to achieve expert levels in a field. • Verbalise: Learning is improved by speaking or verbalising material, as a variation to the more common reading and writing. Try talking through the steps to complete a task. Check assessment tasks by reading them out loud.	• Feedback: This is a powerful tool for improvement. Carefully read feedback comments on your work. If you need more clarification, discuss the feedback with the teacher. Understanding and internalising comments on your work helps you learn from your mistakes. It also helps you look more critically at your own work and develop self-evaluation skills.

TABLE 1.1.1 continued

	Attitudes	Behaviours	Metacognition
Strategies continued	• Concentration, persistence and engagement: Learning is enhanced with concentration and sustained application. Create a study area that minimises distractions. The quality of study time has a greater impact on achievement than the length of time you spend studying. • Perception: Think of difficult tasks as challenges to be tackled and overcome. See mistakes as an opportunity to improve.	• Seek assistance from classmates and/or teachers: Regularly work with a partner or in a group to discuss learning issues and subject matter. Learning is enhanced when you explain material to others. • Summarise subject matter: Creating concept maps of key subject matter is an effective way of summarising material, as are other graphic organisers. • Study skills, note-taking, underlining and highlighting: These are three effective examples of study skills. Use these strategies throughout the year to identify key ideas and enhance understanding. • Memorisation and using mnemonics: Some material simply has to be memorised, such as formulas and key glossary definitions. Using mnemonics may help, as might making charts to summarise key information.	• Reciprocal teaching: Students become the teachers. This is a very effective way of improving understanding by putting yourself in an active role rather than being the passive recipient of knowledge. Create a study group of classmates. Rotate between students, as each takes a turn at teaching (revising) a concept or skill to the others. • Evaluation and reflection: Try assessing your own work against set criteria, such as syllabus ISMGs. This requires an understanding of task objectives and evaluation of whether the criteria are met. This fosters your growth as an independent learner who can guide their own learning. Spend some time reflecting on what you did well and what you need to focus on for improvement, and think about what actions you can take to make those improvements.

LEARNING IN BIOLOGY

Your experience and understanding of science would have developed during the junior years of science lessons and from your observations in the world. Your understanding comes from scientific experimentation, conducting investigations, practicals and learning from your textbooks and teachers.

Science is an evolving body of knowledge that can be captured and explained using different tools, such as physical and mathematical models, diagrams, explanations and equations. These tools, which can be referred to as representations, help build meaning and deepen your understanding of the science you are studying.

To help you improve your learning, it is useful to identify the role of the different representations and the level at which they are used in order to explain scientific concepts. These representations can be simplified into different categories. Table 1.1.2 lists the four categories in biology and provides examples.

TABLE 1.1.2 Representation levels in biology

Representation level	Explanation
macroscopic	observable phenomena where biological structures are visible to the naked eye (e.g. animals and plants)
microscopic	cellular or subcellular level at which structures are visible only with a light or electron microscope (e.g. cells of animal and plant tissue)
submicroscopic	images and illustrations used to show the properties or characteristics of phenomena at a molecular level (e.g. DNA and proteins)
symbolic	figures, numbers or symbols used primarily in mathematical equations (e.g. inheritance patterns and metabolic pathways)

BARRIERS TO LEARNING

In education, factors that prevent a person from learning something effectively are called 'barriers to learning'. There are many different barriers to learning, ranging from language barriers to learning difficulties. Everyone is capable of learning, but finding the techniques and strategies that help to break down these barriers can be a challenge. Each learner has their own preferences about the way they learn, so the barriers differ. Within the more abstract disciplines of science, such as biology, there are additional barriers that come with the subject matter and result in further challenges to learning.

To learn biology effectively, you need to identify your personal barriers to learning and work towards overcoming them. Common barriers to learning in biology are explained for effective learning and building of connections and meaning.

Scientific literacy

The term 'scientific literacy' refers to the ability to make sense of and understand the scientific world in order to make informed decisions. This means having knowledge and understanding of scientific concepts and processes in order to make decisions. Literacy also applies to learning and being able to correctly apply new scientific terms in order to both understand and communicate scientific information. This includes being able to use different representations to deepen your understanding. In other words, being scientifically literate means being able to operate competently like a scientist.

Multiple representations in biology

Biologists investigate animals and plants and how they interact with each other. This can be done by observing animal and plant behaviour. Biologists also talk about phenomena and objects that we cannot see; for example, finding out why fish are dying in a previously clean river. Biology requires the use of imagination and the ability to visualise the underlying cause of this problem. To help with this visualisation, teachers and biologists often produce representations of complex concepts, such as chemical reactions that are causing the pollution.

Visual aids usually take the form of symbols, diagrams or animations that symbolise a concept (Figure 1.1.1). Developing a good understanding of biology requires you to be able to effectively interpret these representations.

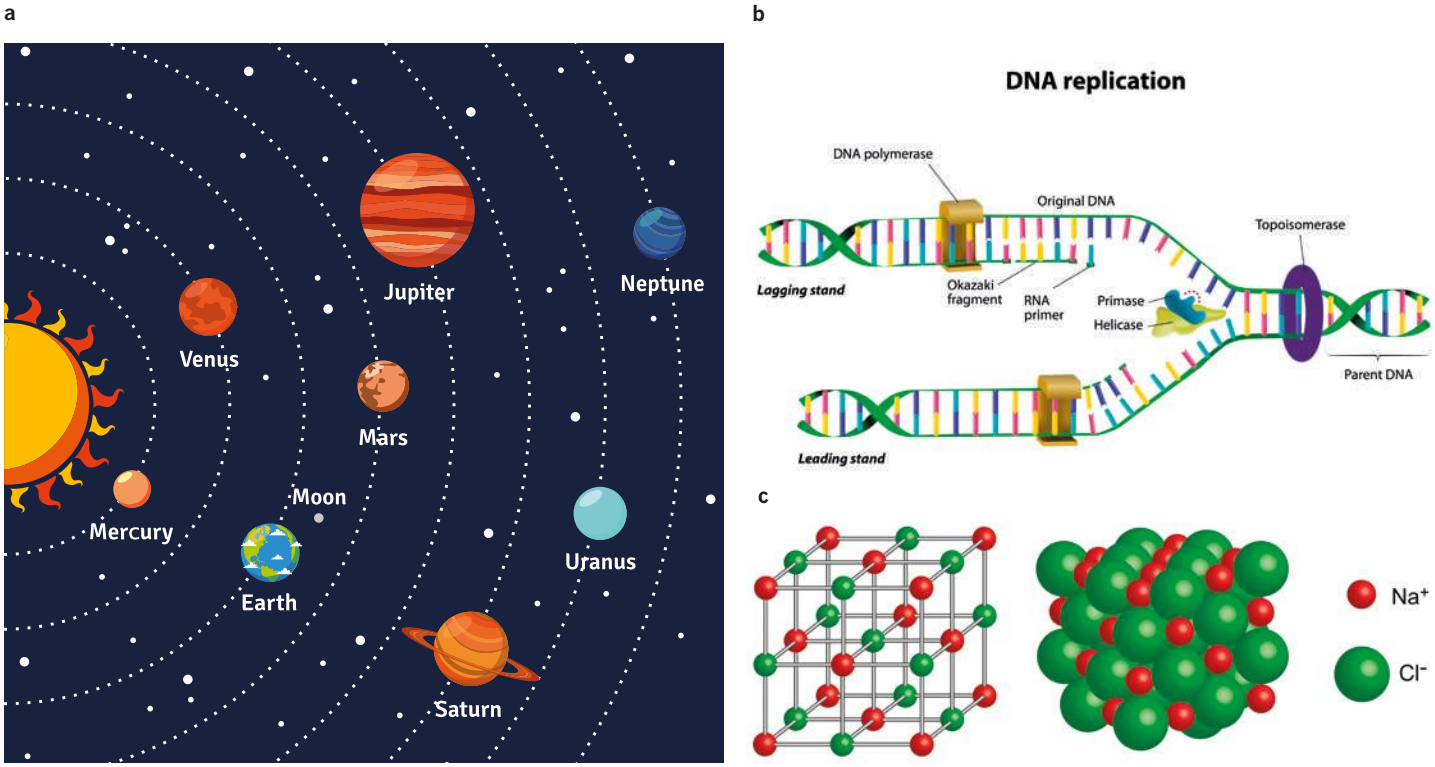


FIGURE 1.1.1 (a) A representation of our solar system (used in physics). (b) A representation of DNA replication (used in biology). (c) A representation showing the formation of an ionic lattice (used in chemistry).

REPRESENTATIONS IN BIOLOGY

Understanding biology requires students to engage with a number of different types of representations. Studies have shown that the key to learning successfully in biology comes down to an awareness and understanding of the representation used to explain and model biological concepts. Scientists have looked at these representations in great detail, and although there are variations in the exact terminology used, scientific studies agree that there are four main levels of representation used in biology, as illustrated in Figure 1.1.2:

1 macroscopic
2 microscopic
3 submicroscopic
4 symbolic.

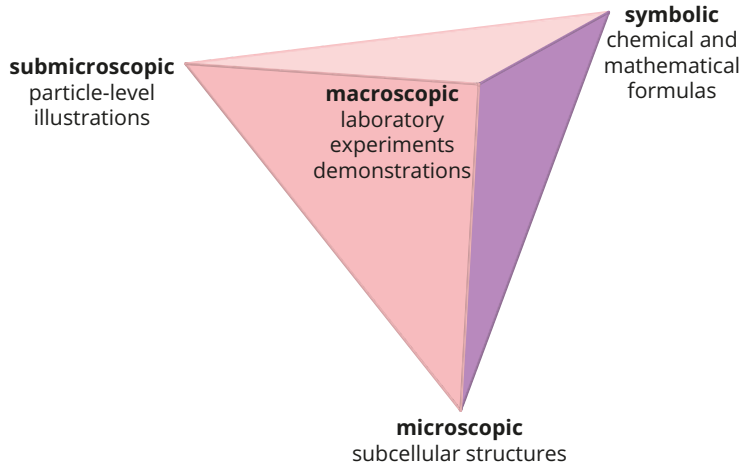


FIGURE 1.1.2 The four levels of representation used in biology.

Macroscopic level

You are probably familiar with the term 'micro' as meaning something very small. 'Macro' is the opposite. It refers to anything that is considered large. In biology, we refer to anything we can observe with our senses or in an experiment as macro, such as growing plants or breeding mice in a classroom. The macroscopic level also focuses on phenomena that can be measured, such as leaf venation patterns or animal body temperature (Figure 1.1.3).

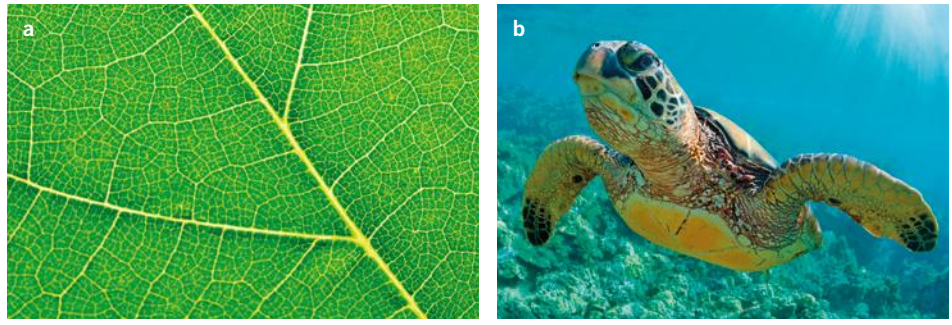


FIGURE 1.1.3 (a) Veins on a leaf and (b) a green turtle are both examples of macroscopic representations.

Macroscopic phenomena can be directly observed and are usually the first way that a concept or idea is experienced, introduced or learnt. Additional and more complex representations are often needed to help explain what is happening at a molecular level. This is where micro, submicroscopic and symbolic representations are used.

Microscopic level

Within biology, there are multiple levels of content organised by hierarchy. For example, cells make up tissues, which in turn make up muscles, which make up organs, which make up organ systems. The microscopic level covers structures that are visible only through a light or electron microscope (Figure 1.1.4).

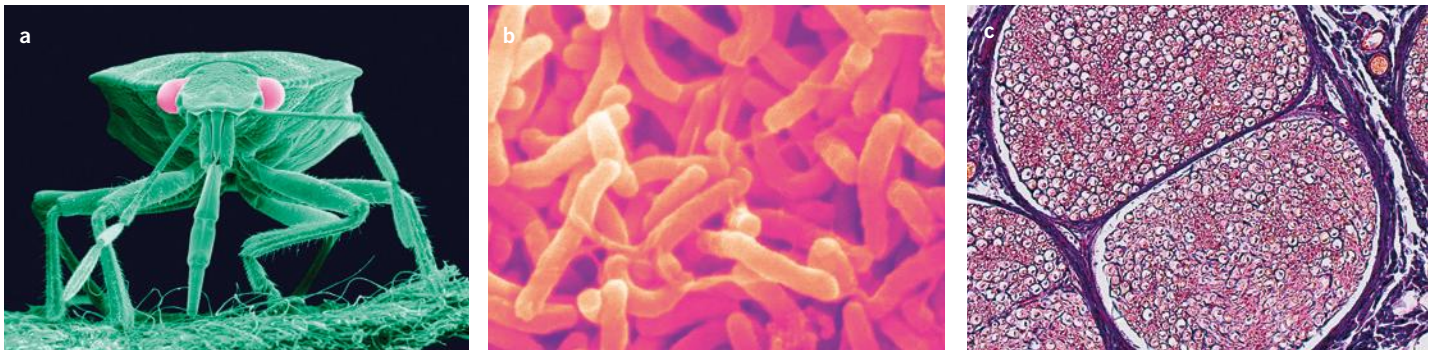


FIGURE 1.1.4 Examples of microscopic representations include (a) scanning electron micrograph of a plant insect, (b) scanning electron micrograph of bacteria and (c) light micrograph of nerve fibres.

Submicroscopic level

Submicroscopic refers to phenomena that are too small to see with a microscope ('micro' means small, and 'sub' means under or beneath). The submicroscopic level is used to represent extremely small biological entities and models are usually used as an aid to understanding (Figure 1.1.5).

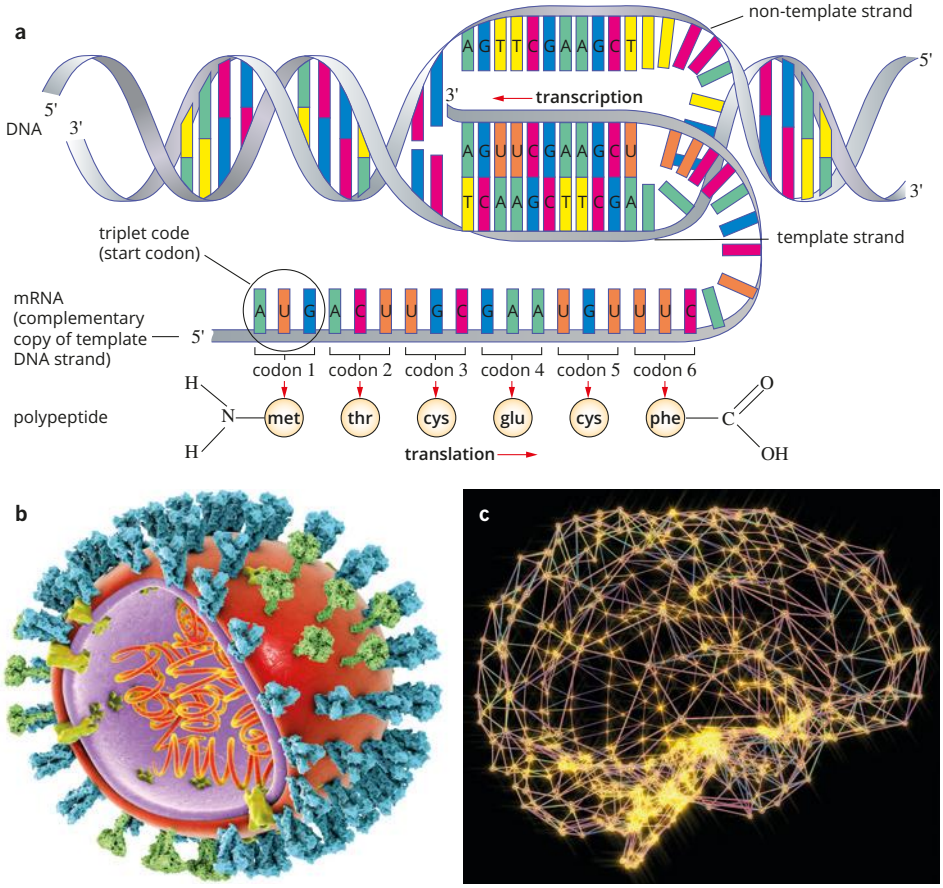


FIGURE 1.1.5 Examples of submicroscopic representations used to explain (a) DNA transcription, (b) the structure of a virus and (c) the brain neural network.

Rather than focusing on observations and measurements, as in the macroscopic level, submicroscopic representations consider properties and characteristics of biological structures to aid understanding of their behaviour or function.

Symbolic level

The symbolic level uses a variety of different symbols and includes everything from mathematical formulas and chemical equations to genotypes and inheritance patterns (Figure 1.1.6).

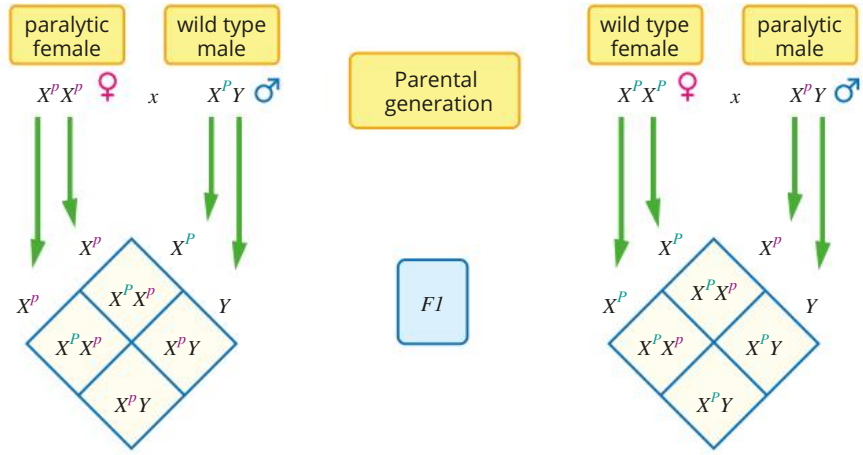


FIGURE 1.1.6 Reciprocal cross diagrams assist in displaying genetic heritance.

While submicroscopic representations focus on properties or characteristics, symbolic representations focus on ways to easily illustrate these properties or characteristics without having to write long passages of text.

Increasing abstraction of representation modes

Within biology, representations take place along a continuum of increasing abstraction, from real life to human language and equations. This continuum can be imagined as a horizontal list, starting at easy-to-understand worldly objects and actions, photographs and animations, natural drawings and maps and diagrams, and moving to more abstract representations such as graphs, tables and equations (Figure 1.1.7).

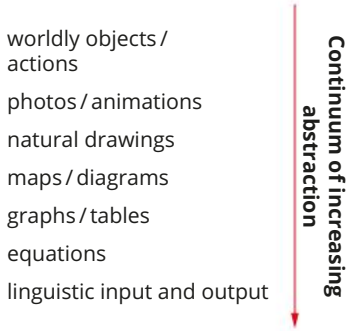


FIGURE 1.1.7 Increasing abstraction of representations used in biology.

Examples of representations of predator–prey relationships could include food webs, photographs and illustrations. More abstract representations, like graphs, require you to interpret information about the predator–prey relationship (Figure 1.1.8).

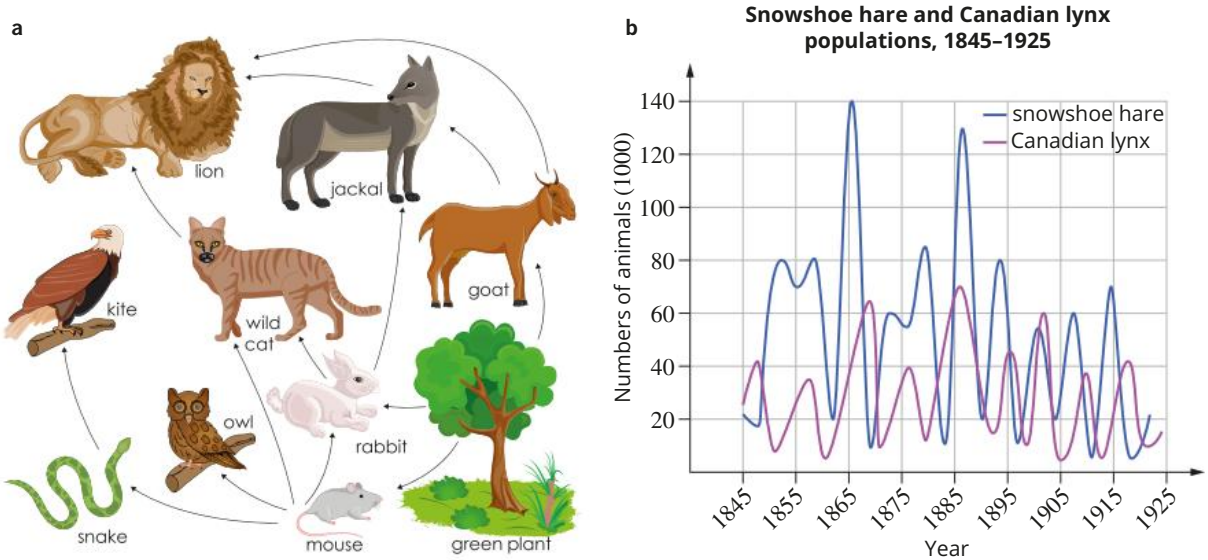


FIGURE 1.1.8 Examples of a predator–prey relationship displayed as (a) a simple food web diagram and (b) a more abstract line graph mode of representation.

USING REPRESENTATIONS TO ENSURE EFFECTIVE LEARNING

This section has emphasised the importance of representations, but also the barriers that they can create for some learners. It is important to be proactive in trying to overcome these barriers and equip yourself with helpful strategies, like concept maps.

To create a concept map, focus on one process or concept, and then branch out into the four ways it can be represented (macroscopic, microscopic, submicroscopic and symbolic). Try to find multiple examples of each representation (Figure 1.1.9). Use a single page to map out the key words and ideas in the topic or chapter. Use link lines to connect words and ideas and explain the connections. This consolidates your learning by representing key concepts in concise ways.

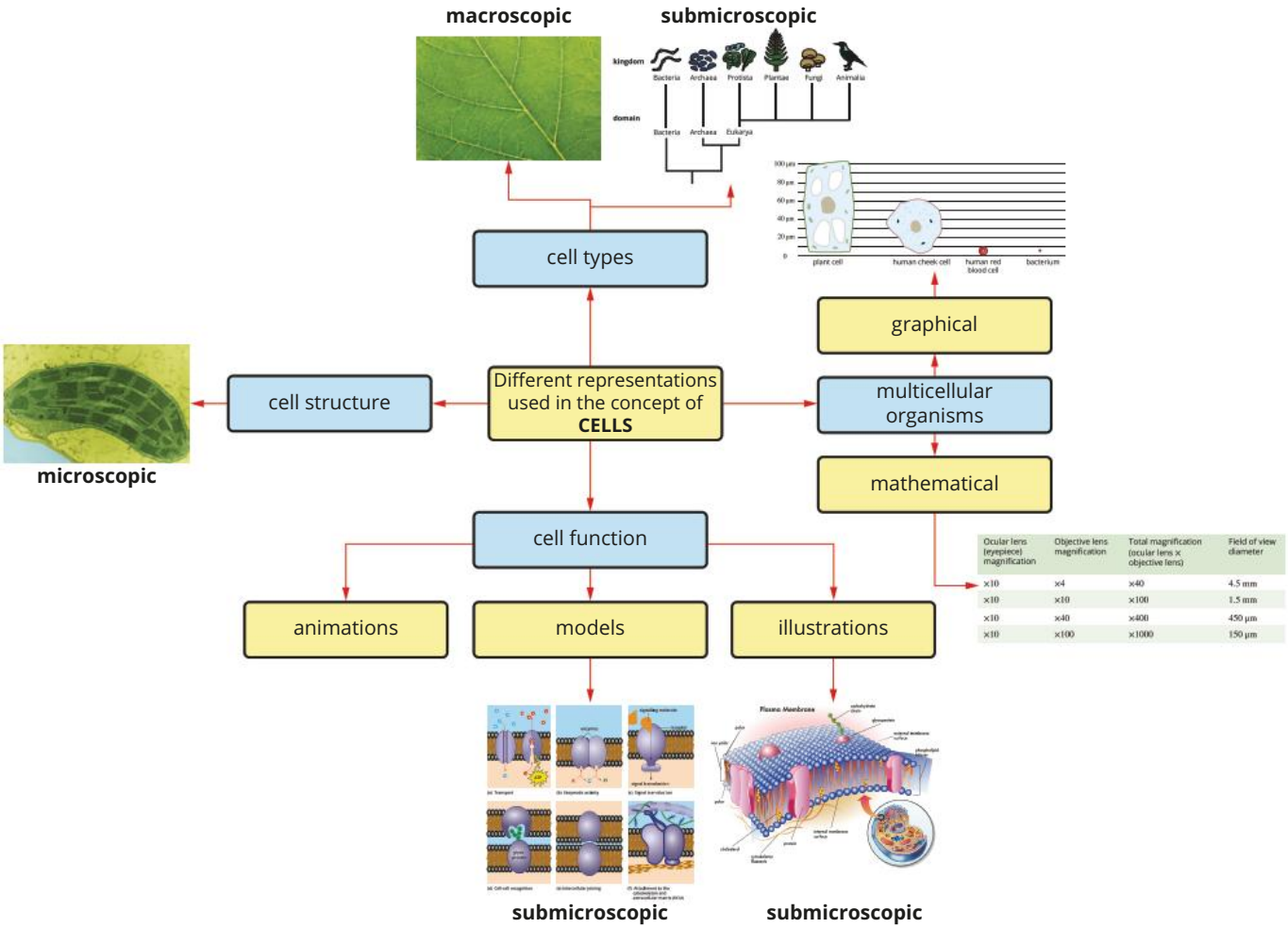


FIGURE 1.1.9 Concept map using the four levels of representation to aid understanding of a biological concept.

You can also use tables to identify gaps in your knowledge. Identify parts of your biology course that you have found difficult and work out which kinds of representations have been used to help explain it. If one is missing, try to create your own. If there is a representation that you do not understand, reflect on what the barrier might be. It could be the terminology used, or a diagram you do not understand. Create your own representation for it. Approach your teacher for assistance if necessary.

In addition to these strategies, this student book contains examples of the different representation levels that you can use. Table 1.1.3 outlines the application and purpose of these features. The list is not comprehensive, and you may find additional examples of features that address the different levels of representations.

TABLE 1.1.3 Representation levels found in the Pearson Biology Queensland series features

Representation level	Pearson Biology series feature	Resource	Application/purpose
literacy	glossary	student book	definitions of key scientific terms
	preliminary material, including Marzano and Kendall's cognitive verbs list and explanation	student book	unpacking meaning behind instructions and enabling understanding of the type of responses required
	literacy review worksheets	skills and assessment book	building language and meaning of discourse terminology
macroscopic	mandatory practicals	student book	requires the use of observation skills to record results
	additional practicals	skills and assessment book	requires the use of observation skills to record results
microscopic	mandatory practicals	student book	requires the use of observation skills to record results
	additional practicals	skills and assessment book	requires the use of observation skills to record results
submicroscopic	diagrams	student book and skills and assessment book	illustrations help to visualise complex chemical processes or concepts
	interactives	Reader+	animations aid with visualising more complex chemical processes or concepts
	videos	Reader+	help illustrate complex chemical processes or concepts
	module, chapter and unit reviews	student book	practice applying learnt terminology in answering questions
symbolic	chemical equations	student book	simpler and often shorter representations of chemical reactions
	structural formulas	student book and skills and assessment book	more efficient ways of illustrating the structure of molecules
	mandatory practicals	student book	requires the use of observation skills to record results
	additional practicals	skills and assessment book	requires the use of observation skills to record results

Teachers and textbooks use biology representations to assist students in their learning. Understanding how these representations are used allows you to identify how you learn best. By identifying the types of representations that cause difficulty in your learning, you can be proactive in overcoming the learning barrier. Use the strategies discussed to ensure effective learning throughout your biology course.

1.1 Review

SUMMARY

- Scientific concepts are expressed by different representations.
- Scientific representations can be placed into four categories: macroscopic, microscopic, submicroscopic and symbolic.
- The macroscopic level deals with observable events in biology.
- The microscopic level deals with objects that can been seen only under a light or electron micrograph.
- The submicroscopic level deals with events that are too small to be seen with a microscope.
- The symbolic level deals with the atomic scale and uses symbols that represent mathematical calculations.
- Effective learning in biology involves understanding the different levels of representations, identifying your own personal barriers to learning and working to overcome them.

KEY QUESTIONS

Retrieval

1 List the different representations used in biology.

Comprehension

2 Demonstrate your understanding of each representation by providing an example of each.

Analysis

3 Reflect on your learning in biology.

a Identify any barriers that block your learning.

b Explain how you might approach overcoming this barrier to your learning.

Part A will consider the basic mathematical skills and applications that will be required for the data test, a range of mandatory and suggested practicals, and the student experiment.

The data test (IA1)

The data test assessment (IA1) is completed at the end of Unit 3 and relates only to material in that part of the biology course. The data test is completed in conditions similar to those of the examination at the end of Unit 4, although the data test's focus is wholly on understanding, analysing and interpreting data and drawing evidence-based conclusions.

The table below outlines some important information about the data test, such as the types of response items, the test conditions and the equipment that can be used.

Data test	
types of items	• short answers: single words, sentences or short paragraphs • longer paragraphs: 50–250 words per item • other types of item responses involving calculations or interpretations
syllabus coverage	• Unit 3
assessment objectives	• apply understanding • analyse evidence • interpret evidence and draw conclusions
conditions	• perusal (reading) time: 10 minutes • writing time: 60 minutes
equipment	• approved graphics calculator • unseen stimulus material
marks	• constitutes 10% of total assessment

Studying for the data test cannot be rushed just before the test. It is not a memory-style test that you can cram for at the last minute. The skills and thinking required to effectively complete the data test are best acquired over an extended period of time, with practice and more practice. Part A outlines many of the skills that will enhance your performance when working with data. Refer to Part A when you need to develop your skills.

1.2 Orders of magnitude and estimation

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- convert values from decimal notation to scientific notation and vice versa
- convert values from scientific notation to scientific units and vice versa
- solve biological calculations that have values in scientific notation or use scientific units.

SCIENTIFIC NOTATION

In science, measurements are often written in **scientific notation**. Quantities are written as a number between 1 and 10 and then multiplied by an appropriate power of 10.

Examples of some values written in scientific notation are:

- 6.022×10^{23} particles
- $25.25\,\text{mL} = 2.525 \times 10^{-2}\,\text{L}$
- $0.00302\,\text{mol} = 3.02 \times 10^{-3}\,\text{mol}$

Values given in scientific notation show the significant numbers and indicate the precision of measurement during experimentation by the instrument. See Module 1.5 for more details about significant figures.

'Scientific notation', 'standard notation' and 'standard form' all have the same meaning.

SKILLBUILDER

Transforming decimal notation to scientific notation

Scientists use scientific notation to deal with very large and very small numbers. For example, instead of writing 0.000 000 035, scientists would write 3.5×10^{-8}.

A number in scientific notation (also called standard form or power of ten notation) is written as:

$$a \times 10^n$$

where

a is a number equal to or greater than 1 and less than 10; that is, $1 \leq a < 10$

n is an integer (a positive or negative whole number)

n is the power that 10 is raised to and is called the index value.

To transform a very large or very small number into scientific notation:

- write the original number as a decimal number greater than or equal to 1 but less than 10
- multiply the decimal number by the appropriate power of 10.

The index value is determined by counting the number of places the decimal point needs to be moved to form a.

- If the decimal point is moved n places to the left, n will be a positive number. For example: $3750 = 3.75 \times 10^3$.
- If the decimal point is moved n places to the right, n will be a negative number. For example: $0.084 = 8.4 \times 10^{-2}$.

This is shown in Figure 1.2.1.

You will notice from these examples that when large numbers are written in scientific notation, the 10 has a positive index value. Numbers less than 1 are written with 10 to a negative index.

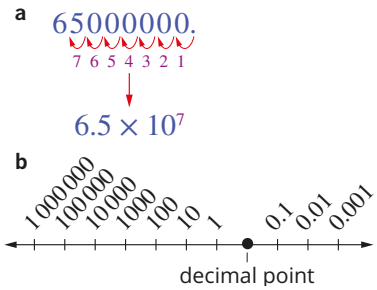


FIGURE 1.2.1 (a) The number of places the decimal moves becomes the power index. (b) Decimal place according to place value. (c) Common numbers in expanded form with their respective scientific notation.

c

Scientific notation	Number (expanded form)
1×10^{-9}	0.000 000 001
1×10^{-6}	0.000 00 1
1×10^{-3}	0.001
1×10^{-1}	0.1
1	1
1×10^{1}	10
1×10^{3}	1000
1×10^{6}	1 000 000
1×10^{9}	1 000 000 000

Worked example 1.2.1

CONVERTING TO SCIENTIFIC NOTATION

The population of black nerite (*Nerita atramentosa*) in a 1 m^2 quadrat in the high mid-littoral zone was counted to be 43. Calculate the total abundance of the black nerite across the high mid-littoral zone of the rocky shore ecosystem, which was measured to be 678 m^2. Report in scientific notation.	
Thinking	**Working**
Multiply the number of black nerites counted per m^2 by the total m^2 of the ecosystem.	$43 \times 678 = 29\,154$
Move the decimal place to the final digit.	29154 = 2.9154
Count the number of places the decimal placed was moved.	4
Use the number of decimal places moved as the power index to multiply 10.	2.9154×10^4
Determine whether the decimal was moved left or right.	left
The power index, 4, is a positive number. Write the answer.	It is estimated that the total abundance of black nerites in the high mid-littoral zone of the rocky shore ecosystem is $2.91\,54 \times 10^4$.

➤ Try yourself 1.2.1

CONVERTING TO SCIENTIFIC NOTATION

A Danish study of 25 individuals showed that 2.5% of the single DNA mutations (substitution) occurred in the place of the same gene. Use this data to infer how many Danish individuals share the same mutation when the population is 5 748 769. Report in scientific notation.

Use scientific notation to display numbers with at least three digits or more.

Scientific and graphics calculators can be put into a mode in which all numbers are displayed in scientific notation. This is useful and less time-consuming when completing calculations than counting the digits on the calculator display to convert to scientific notation.

Using scientific notation removes ambiguity about the precision of some measurements. For example, a measurement recorded as 240 g could be a measurement to the nearest gram—somewhere between 239.5 and 240.5 g—or it could have been rounded up to the nearest 10 g. Writing this quantity as 2.40×10^2 g makes the accuracy of the value clear. In this case, it is three significant figures. (See Module 1.5 for further explanation.)

ORDERS OF MAGNITUDE AND PREFIXES

Various scientists (for example, physicists, chemists and biologists) work with very large or very small numbers. It is important to understand the numbers in scientific text and their order of magnitude and be able to convert them into other units for scientific analysis and interpretation.

Displaying numbers with magnitude can be achieved with scientific notation or by using appropriate units. For example, 2.56×10^{-6} m can be written in scientific notation, as it is already, or as 256 µm. The symbol µ represents the prefix micro, which means one-millionth of the measured unit. When micro is placed in front of metre it becomes micrometre, meaning one-millionth of a metre. Therefore, 256 µm means 256 millionths of a metre.

Each prefix in Table 1.2.1 represents an order of magnitude and you should be familiar with both scientific notation and prefixes for units of measurement. You should also be able to interchange them by referring to Table 1.2.1.

TABLE 1.2.1 The 12 official SI prefixes used in biology

Prefix	Symbol	Multiplier	Description	Decimal	Example
tera	T	10^{12}	trillion	1 000 000 000 000	1 TByte hard drives are now common.
giga	G	10^{9}	billion	1 000 000 000	3.16 Gs = 1 century.
mega	M	10^{6}	million	1 000 000	1 MHz is close to the frequencies at which TV stations broadcast.
kilo	k	10^{3}	thousand	1000	1 kg is 1000 grams.
hecto	h	10^{2}	hundred	100	1 hPa is a unit used in meteorology to describe atmospheric pressure.
deca	da	10^{1}	ten	10	1 daN is approximately the force exerted by a 1 kg object on the surface of the Earth.
–	–	1	unit	1	–
deci	d	10^{-1}	tenth	0.1	1 L = 1 dm^3
centi	c	10^{-2}	hundredth	0.01	1 cm^3 is equivalent to 1 mL.
milli	m	10^{-3}	thousandth	0.001	There are 1000 mm in 1 m.
micro	µ	10^{-6}	millionth	0.000 001	µg is the unit commonly used for medicine dosage.
nano	n	10^{-9}	billionth	0.000 000 001	The wavelength of yellow sodium streetlights is 550 nm.
pico	p	10^{-12}	trillionth	0.000 000 000 001	Blood concentrations of insulin are measured in picomoles (pmol).

SKILLBUILDER

Converting between units

Often in science you need to know how to convert from one unit to another to complete a calculation. It is important to know the size of different units in relation to each other to avoid errors in calculations. It is important to give a symbol the correct case (upper or lower case)—there is a substantial difference between 1 mm and 1 Mm.

For example, blood concentrations of insulin can be measured in picomoles (pmol). Some studies have measured the amount of insulin in blood after a meal of pasta to be approximately 40 pmol L^{-1}. To put 40 pmol L^{-1} into context, you can convert this value into a unit you can easily visualise, such as grams per litre (g L^{-1}).

First convert 40 pmol L^{-1} to mol L^{-1} by moving the decimal 14 places to the left, so it becomes 0.000 000 000 0004 mol L^{-1}. This can also be written as 4.0×10^{-14} mol L^{-1}. One mole of insulin weighs 5800 g; therefore, 0.000 000 000 000 4 mol L^{-1} is equal to 0.000 000 232 g L^{-1} (or 2.32×10^{-7} g L^{-1}), which is very small indeed!

Worked example 1.2.2

CONVERTING BETWEEN UNITS

The rate of photosynthesis is measured in some plants by measuring the change in atmospheric CO_2 in moles per m^2 of leaf area, per second (mol/m^2/s or mol $m^{-2}s^{-1}$). If a plant was measured to photosynthesise CO_2 at a rate of 25 µmol $m^{-2}s^{-1}$, calculate how many moles of CO_2 the plant would photosynthesise per second, and then per hour.	
Thinking	**Working**
To calculate moles per second, multiply 25 µmol by the multiplying factor for µ (micro).	$25 \times 0.000\,001 = 0.000\,025$ mol $m^{-2}s^{-1}$
To calculate moles per hour, multiply 0.000 025 mol $m^{-2}s^{-1}$ by 3600 (because there are 3600 s in an hour).	$0.000\,025 \times 3600 = 0.09$ mol $m^{-2}h^{-1}$

➤ Try yourself 1.2.2

CONVERTING BETWEEN UNITS

The photosynthetic rate of CO_2 by a plant was measured to be 6.2 µmol $m^{-2}s^{-1}$. Calculate the number of moles of CO_2 that are photosynthesised per square metre per second.

Worked example 1.2.3

CONVERTING BETWEEN UNITS WITH MAGNITUDE

A bacterial culture contains 2×10^{11} cells L^{-1}. If you transferred 2 mL of the bacterial culture to an agar plate, calculate how many bacterial cells you transferred. Provide the answer in scientific notation.	
Thinking	**Working**
To convert cells L^{-1} to cells mL^{-1}, multiply 2×10^{11} by the multiplying factor for milli (m).	$2 \times 10^{11} \times 0.001 = 2 \times 10^8$
Multiply 2×10^8 cells mL^{-1} by 2.	$2 \times 10^8 \times 2 = 4 \times 10^8$ cells mL^{-1}

➤ Try yourself 1.2.3

CONVERTING BETWEEN UNITS WITH MAGNITUDE

Catalase in liver cells can convert 4×10^7 molecules of hydrogen peroxide to oxygen and water per second. The activation energy of a catalysed reaction controlled by catalase is 6.58×10^6 J mol^{-1}. Convert this to kJ mol^{-1}.

1.2 Review

SUMMARY

- 'Scientific notation', 'standard notation' and 'standard form' all have the same meaning.
- A number in scientific notation (also called standard form or power of ten notation) is written as $a \times 10^n$:

  where
  - a is a number greater than or equal to 1 and less than 10, i.e. $1 \leq a < 10$
  - n is an integer (a positive or negative whole number)
  - n is the power that 10 is raised to and is called the index value.
- A range of scientific symbols are used to represent 10^{3n}.

KEY QUESTIONS

Retrieval

1 Recall the prefix and symbol for the following orders of magnitude.

a 1 000 000 000
b 1000
c 0.000 001
d 0.000 000 001
e 0.001

Comprehension

2 Convert the following to scientific notation.

a 0.000 000 000 012
b 0.000 45
c 73 000 000 000 000 000
d 214
e 16 201

3 Convert the following to scientific notation.

a 1.7 nm using m
b 8.2 TW using W
c 2.4 ML using L

Analysis

4 A typical household plant produces 5 mL h^{-1} of oxygen. Sally has 12 plants in her home and decided to calculate how many litres of oxygen they produce per week collectively. She estimated they produced oxygen for 12 h d^{-1} and calculated a total of 5040 L wk^{-1}. Sally's calculation is incorrect. Identify where she made an error.

5 Grassland consumption of 3360 kg/hectare/year of CO_2 was converted to 920.5 g/hectare/day (four significant figures). Identify where the error occurred in the conversion.

1.3 Mathematical basics for biology

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand that the rules of mathematics apply for solving mathematical operations or calculations in biology.

Science uses mathematics in working with data. The evidence can be quantified, measured and analysed. This is known as statistical analysis (a branch of mathematics). The mathematical processing and statistical analysis is required for the evidence and science to be interpreted.

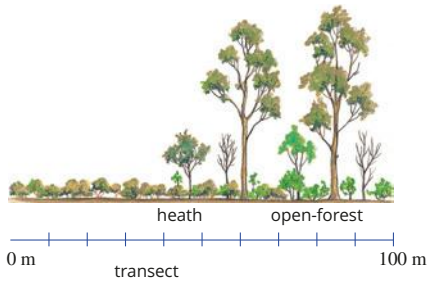
When conducting mathematical operations or calculations, remember that all the rules of mathematics apply. These include:

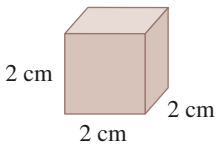
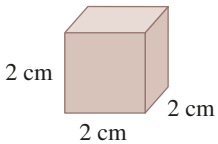
- order of operations
- rules for transposing formulas and working with fractions
- substitutions
- approximations.

EXAMPLES OF MATHEMATICAL BASICS IN BIOLOGY

Table 1.3.1 shows some examples of the use of mathematics in biology.

TABLE 1.3.1 Examples of mathematics in biology

Calculating the size of an area (quadrat)	
	$A = S^2$ $= 0.5^2$ $= 0.25\,m^2$
Calculating the size of an area (transect line—rectangular)	
100 m transect line, sampled every metre with a 1 m quadrat on both sides of the transect line  	$A = L \times W$ $= 100 \times 2$ $= 200\,m^2$
Calculating the mean abundance per m^2	
1 m quadrat sampling the number of weeds 	Count the number of specimens for each quadrat (Q) sampled: Q1 = 5 Q2 = 3 Q3 = 6 Q4 = 4 Q5 = 4 Calculate the mean abundance per sampled area (m^2). $\text{mean} = \frac{n1 + n2 + n3 + n4 + n5}{N}$ $= \frac{5 + 3 + 6 + 4 + 4}{5}$ $= 4.4\,m^2$

Calculating the population of a species	
Sampled area of 54 × 185 m sheep grazing farmland 	(using the weed samples above) Population = abundance (per sampled area) × total area Population = 4.4 × (54 × 185) = 4.4 × 9990 = 43 956 weeds
Calculating species richness	
Rocky shore quadrat, displaying all the organisms within the sample quadrat 	Quadrat abundance counts: Species 1 = 3 Species 2 = 4 Species 3 = 1 Species 4 = 64 species richness (D) = $\frac{s}{\sqrt{N}}$ $= \frac{4}{\sqrt{72}}$ = 0.47
Calculating Simpson's diversity index	
Rocky shore quadrat, displaying all the organisms within the sample quadrat 	Quadrat abundance counts: Species 1 = 3 Species 2 = 4 Species 3 = 1 Species 4 = 64 Simpson's diversity index (SDI) $\text{SDI} = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$ $= 1 - \left(\frac{3(3-1)+4(4-1)+1(1-1)+64(64-1)}{72(72-1)}\right)$ $= 1 - \left(\frac{4050}{5112}\right)$ = 0.21
Calculating surface area (SA)	
	Dimensions of cells (or simulated cells) are required when determining a cell's capacity to undergo diffusion. SA = (length × length) × 6 sides = (2 × 2) × 6 = 24 cm^2
Calculate surface area to volume ratio (SA : vol)	
	This is required to understand the relationship between cell size and rate of diffusion. This also helps to understand the structure and function relationship in cells. SA : vol = $S^2 \times 6 : S^3$ = $2^2 \times 6 : 2^3$ = 24 : 8 = 3 : 1

1.3 Review

SUMMARY

- When conducting mathematical operations or calculations, remember all the rules of mathematics apply, including:
  - order of operations
  - rules for transposing formulas and working with fractions
  - substitutions
  - approximations.

KEY QUESTIONS

Retrieval

1 List three ways that mathematics can assist in the study of biology.

Comprehension

2 Calculate the mean number of individuals sampled across 6 quadrats, with the number of individuals in each quadrat as follows: 7, 9, 13, 8, 8, 9.

3 Calculate the size of the sample area for 2 m × 2 m quadrats sampling every 2 m along a 12 m transect line.

4 Calculate the surface area to volume ratio of a rectangle measuring 3 µm × 3 µm × 15 µm.

5 Calculate Simpson's diversity index for the following data:
Quadrat abundance counts: Species A = 9, Species B = 2, Species C = 24, Species D = 41.

Analysis

6 Argue what would happen to the SA : vol ratio if the volume of a shape increased.

7 Calculate which class would sample a larger area: class A sampling every 3 m along a 24 m transect line with a 1 m × 1 m quadrat, or class B sampling every 4 m along a 24 m transect line with a 1 m × 2 m quadrat.

1.4 Units

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- appreciate that biology uses the International System of Units (SI)
- recall some common SI units and symbols used in biology
- write measurements and communicate quantities using SI units
- understand that the SI prefixes are multipliers of powers of ten of the base units
- convert between various units of the same type, using SI and some non-SI units.

MEASUREMENT AND UNITS

Scientists use conventional ways of writing numerical measurements, such as the International System of Units (SI). Use common or SI units when recording your evidence. Table 1.4.1 presents common quantities and SI units used in biology.

TABLE 1.4.1 Common quantities and SI units used in biology

Quantity	SI unit (symbol)	Common unit (symbol)
mass	kilogram (kg)	gram (g)
volume	litre (L)	millilitre (mL) decilitre (dL)
energy	joule (J)	kilojoule (kJ)
concentration	moles per litre (mol L^{-1})	grams per decilitre (g dL^{-1}) grams per 100 mL (g 100 mL^{-1})
per cent solution		weight per volume (w/v) weight per weight (w/w)
temperature	kelvin (K)	Celsius (°C)
time	second (s)	minute (min)

Correct use of unit symbols

SI units are recognised internationally so it is important to use the symbols correctly to avoid ambiguity. Table 1.4.2 lists some important points to remember when writing units and recording measurements and numbers. Some examples of the use of symbols for derived units are listed in Table 1.4.3.

TABLE 1.4.2 Examples of correct and incorrect use of symbols for derived units

Rule	Correct	Incorrect
Do not mix written numbers and symbols.	thirty grams 30 grams 30 g	thirty g
Do not put spaces between prefixes and unit symbols.	kg mL	k g m L
Use the correct case for symbols (upper or lower case).	1 mm 84 kg 5.2 mL	1 Mm 84 KG 5.2 ml
Do not put a full stop after SI units unless they are at the end of a sentence. The symbols for units are not abbreviations.	17 mg	17 m.g. 17 mg.

TABLE 1.4.3 Examples of the use of symbols for derived units

Correct symbol	Incorrect symbol
g/dL g dL^{-1}	gm/dl gdL^{-1}
g/kg/day g kg^{-1} day^{-1}	gm/Kg/day $gkg^{-1}day^{-1}$

TABLE 1.4.2 continued

Rule	Correct	Incorrect
Only use upper-case letters for the symbols of the units that are named after people. The exception to this rule is 'L' for litre, where there may be confusion because 'l' looks like the numeral '1'.	110 J (the unit of energy is joule, named after James Joule)	110 j
Units named after people can take the plural form by adding an 's' when used with numbers greater than one. Never do this with the unit symbols.	two kilojoules	2 kJs
Show the product of a number of units by separating the symbol for each unit with a space. The division or ratio of two or more units can be shown in fraction form, using a slash or negative indices.	3.2 kW h 1.7 W/h 1.7 W h^{-1}	3.2 kWh 1.7 W/h^{-1}
When units are displayed with a negative exponent such as g dL^{-1}, the negative value of the exponent signifies the division of the units, that is, per unit. In this case, g dL^{-1} is the same as g/dL, or grams per decilitre.	40 g dL^{-1} 11.6 g h^{-1}	40 g/dL^{-1} 11.6^{-1} g h

1.4 Review

SUMMARY

- Scientists use conventional ways of writing numerical measurements such as SI units.
- Common quantities and SI units used in biology include:

Quantity	SI unit (symbol)	Common unit (symbol)
mass	kilogram (kg)	gram (g)
volume	litre (L)	millilitre (mL) decilitre (dL)
energy	joule (J)	kilojoule (kJ)
concentration	moles per litre (mol L^{-1})	grams per decilitre (g dL^{-1}) grams per 100 mL (g 100 mL^{-1})
per cent solution		weight per volume (w/v) weight per weight (w/w)
temperature	kelvin (K)	Celsius (°C)
time	second (s)	minute (min)

- When units are displayed with a negative exponent, the negative value of the exponent signifies the division of the units. For example, g mL^{-1} is the same as g/mL or grams per millilitre.
- When writing and recording measurements, remember:
  - do not mix numbers and symbols
  - do not put spaces between prefixes and unit symbols
  - write the correct case for symbols (upper or lower case).

KEY QUESTIONS

Retrieval

1 Recall the SI unit and symbol for:
- **a** time
- **b** temperature
- **c** mass

Comprehension

2 Explain why it is important that general society, not just the scientific community, use a systematic and consistent set of units for measurement.

3 Describe the error with the units of 32 g dL^{-1} (g dL^{-1} represents grams per decilitre).

Analysis

4 Identify and correct any errors in the following measurements.
- **a** 23 mL/g h^{-1}
- **b** 79 Kl s^{-1}
- **c** four litres
- **d** 1.56 grams

1.5 Uncertainties in measurement and error

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- explain the difference between accuracy and precision
- explain the difference between uncertainty and error
- calculate uncertainty and error values for measurements
- write measurements with uncertainty and error values
- use the correct number of significant figures
- perform calculations that contain uncertainty or error values (an important skill for assessments).

All measurements are limited by both the instrument used and the researcher, and the biology syllabus expects significant limitations to be identified in assessments. These limitations affect the accuracy of the results. Instruments have a designed level of precision and cannot measure within the smallest increment of the device. The researcher subjectively influences the accuracy of the measurement by, for example, their perspective of concepts, level of experience, choice of variables to control and ability to measure, and choice of population to sample. In science, it is impossible to measure a **true value**.

Errors and uncertainty in the values measured or observed are always present. As such, science interprets results with limitations using a systematic approach that can be scrutinised and validated. Scientific measurement always produces a **range** (e.g. ± 0.25) in the data, indicating possible accuracy. This can also be estimated by processing data through statistical calculations.

ACCURACY AND PRECISION

In science and statistics, the terms 'accuracy' and 'precision' have very specific and different meanings.

- **Accuracy** is the ability to obtain the correct measurement. To obtain accurate results, you must minimise **systematic errors**.
- **Precision** is the ability to consistently obtain the same measurement. To obtain precise results, you must minimise **random errors**.

Figure 1.5.1 (page e28) will help you to understand the difference between accuracy and precision. Accuracy is being able to hit the bullseye (true value). Precision is being able to hit the same spot every time you shoot (consistency). Measurements should be both as precise and accurate as you can make them.

Recording numerical data

When using instruments to measure, the number of significant figures (or digits) and decimal places you record is determined by the precision of the instrument (Figure 1.5.2 on page e28). The precision determines the significant figures for the processed data.

A demonstration of instrumental precision determining significant figures is the use of a beaker (±5 mL) to measure volumes, compared to a graduated pipette (±0.5 mL). The increments are much smaller on the pipette and therefore can more accurately measure the same volume consistently. Such graduated pipettes enable more significant figures to be recorded than a beaker for the same volume measured.

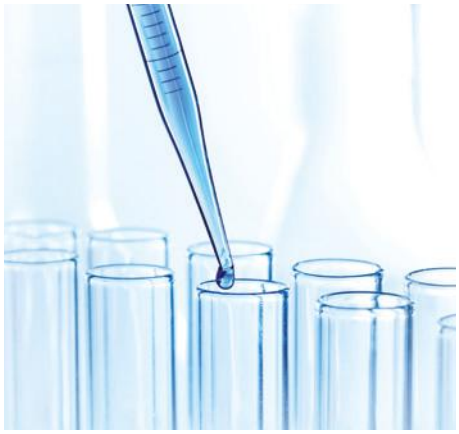


FIGURE 1.5.2 A 5 mL graduated pipette can measure volumes to an accuracy of one-twentieth of a millilitre, or 5 mL ± 0.05 mL. The pipette has major divisions of 1 mL and minor divisions of 0.1 mL. You can measure and record volumes to one decimal place within 0.05 mL either side, for example, 3.8 mL ± 0.05 mL.

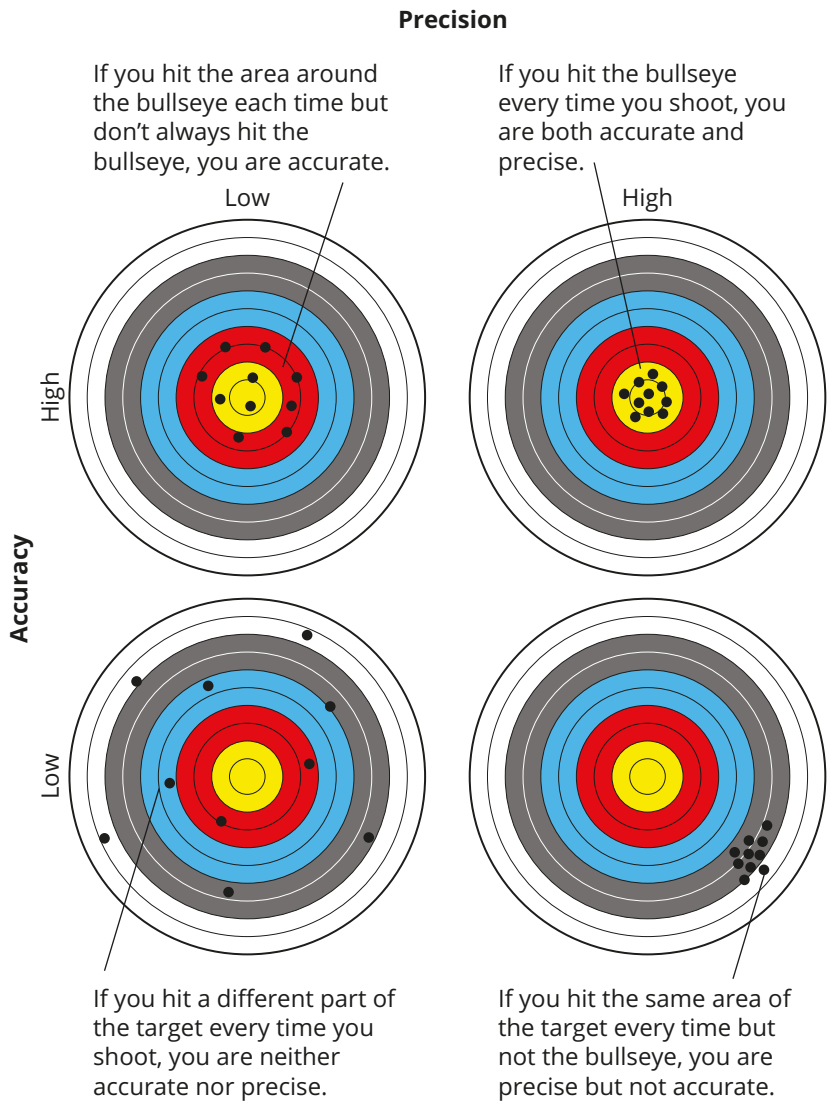


FIGURE 1.5.1 The difference between accuracy and precision.

When you record **raw data** and report **processed data** (Module 1.9), use the number of significant figures available from your equipment or observation. Using either a greater or a smaller number of significant figures can be misleading. For example, Table 1.5.1 shows measurements of five seedling samples taken with an electronic balance that is accurate to two decimal places. The data was entered into a spreadsheet to calculate the **mean**, which was displayed with four decimal places. You would record the mean as 20.83 g, not 20.8260 g, because two decimal places is the precision limit of the instrument. Recording 20.8260 g would be an example of false precision.

TABLE 1.5.1 An example of false precision in a data calculation

Sample	1	2	3	4	5	mean
Mass (g)	20.13	20.62	21.22	20.99	21.17	20.8260

UNCERTAINTY AND ERROR

Uncertainty and errors arise from various sources.

Uncertainty is the range applied to measurements within which the true value is thought to lie. As there are always variations in measurements, an estimate is made to suggest where the true value should be found among the measurements. This quantifies the uncertainty and is one source of limitation.

Error is the difference between a measurement and the true value. This is not an indication of human error or mistakes. It is due to an inability to achieve the true value. Error cannot be eliminated; therefore, it is important to understand and record the error and report it.

Two types of errors can occur—systematic error and random error. These are explained in Table 1.5.2.

TABLE 1.5.2 Systemic and random errors

Error	Definition	Cause	Example
systematic	An error in measurements caused by the design of a system (e.g. methodology) or an instrument (non-calibrated), which results in the measurements shifting in a systemic direction. The mean may be displaced or varied in a predictable way, affecting the accuracy of the measurement.	• instrument calibration • choice of instrument • precision of instrument (increments) • precision of instrument (sample rate per second) • sampling (the choice of population or representatives)	If a digital probe is used to measure the pH of various soil samples, and it was not calibrated before the experiment or between testing, then the measured pH will all be shifted equivalently.
			During an ecological study, choosing to place a quadrat in the areas where it is quickest and easiest to count species. This methodology will cause the recorded results to consistently show lower numbers that are further from the true value than random sample selection, resulting in a systematic downward shift in results.
random	An error that affects the measurement in an unpredictable way, which results in an even variation (fluctuation) in measurements above and below the expected true value. This affects the precision of the measurement.	• estimation (measuring between increments) • sampling (frequency, spread, increments) • parallax • instrument sensitivity • noise	Using a universal indicator to determine the pH of a solution, in which the pH must be estimated by the shade and colour of the paper (e.g. yellow/orange versus bright orange).
			Using a digital probe to measure gas pressure or concentration once per 10 seconds compared with a probe that measures 5 times per second.

Uncertainties and errors should always be calculated or measured and then reported. They then form part of the analysis and interpretation (Part B, Module 1.10 Results, Tables 1.10.1–3) as well as the evaluation of the investigation. Also, it is a good idea to consider uncertainty and error when planning experiments (Module 1.8 and Module 1.9).

Calculating uncertainty in measurement

The goal when taking measurements is to get as close as possible to the 'true' or 'correct' value. This may be challenging if the reading is between two increments on an instrument and the exact value cannot be determined. Uncertainty can be calculated, or estimated, to determine where the true value lies and can be represented as a percentage or unit of measurement. If the uncertainty was described as ±0.05 mg, it means that the measurement taken is likely to be no more than 0.05 mg above or below the 'true' value.

Uncertainty can be understood in three ways:

- uncertainty—a general acknowledgement that there are variations in a measurement
- absolute uncertainty—which acknowledges the accuracy of the instruments being used to measure
- relative uncertainty—which is useful to compare the reliability of trials or sets of measurements against one another.

When using sets of data or repeated measurements and then displaying an average, the uncertainty should be reported alongside your average. One simple way to calculate the uncertainty is:

$$\text{uncertainty} = \pm\frac{\text{maximum value} - \text{minimum value}}{2}$$

Worked example 1.5.1

CALCULATING UNCERTAINTY

An experiment was conducted to measure the biodiversity within a habitat. Three scientists counted the number of individual organisms for species A within one quadrat, recording numbers of 21, 23 and 25. Calculate the uncertainty and report it with the mean.

Thinking	Working
Write down the formula.	$\text{uncertainty} = \pm\frac{\text{maximum value} - \text{minimum value}}{2}$
Insert the values then perform the calculation.	$\text{uncertainty} = \pm\frac{(25 - 21)}{2}$ $= \pm 2$
Calculate the mean of individual organisms of species A in the quadrat.	$\text{mean} = \frac{\Sigma n}{N}$ $= \frac{69}{3}$ $= 23$
Report the uncertainty with the mean.	23 ± 2

➤ Try yourself 1.5.1

CALCULATING UNCERTAINTY

Five students observed the number of different species within a quadrat and each recorded their observations. The recorded numbers were 13, 15, 11, 13 and 14. Calculate the uncertainty and report it with an estimate of the number of species in the quadrat.

Another example of uncertainty is instrumental error or uncertainty in measurement. This is the precision of an instrument and is limited by the smallest increment the instrument can measure. The uncertainty of an instrument is half the smallest increment. This is known as **absolute uncertainty**. A simple way to calculate the absolute uncertainty of a measurement is:

$$\text{absolute uncertainty} = \pm\frac{\text{smallest increment}}{2}$$

Worked example 1.5.2

CALCULATING ABSOLUTE UNCERTAINTY FOR INSTRUMENTAL ERROR

The ambient temperature measured at 10.36 am during a transect survey was 32°C. Both a glass thermometer and a digital thermometer were used. The smallest increment on the glass thermometer was 1°C and the smallest measurable increment on the digital thermometer was 0.1°C. Calculate the absolute uncertainty of both instruments.	
Thinking	**Working**
For the glass thermometer, write down the formula.	$\text{absolute uncertainty} = \pm\frac{\text{smallest increment}}{2}$
Insert the values then perform the calculation.	$\text{absolute uncertainty} = \pm\frac{1}{2}$ $= \pm 0.5$
Combine the measurement with the uncertainty.	32 ± 0.5°C
For the digital thermometer, write down the formula.	$\text{absolute uncertainty} = \pm\frac{\text{smallest increment}}{2}$
Insert the values and perform the calculation.	$\text{absolute uncertainty} = \pm\frac{0.1}{2}$ $= \pm 0.05$
Combine the measurement with the uncertainty.	32 ± 0.05°C

➤ Try yourself 1.5.2

CALCULATING ABSOLUTE UNCERTAINTY FOR INSTRUMENTAL ERROR

A group of students surveying an ecosystem measured the wind speed with an anemometer at 25.8 $km\,h^{-1}$. Calculate the absolute uncertainty for the wind speed.

Uncertainty can also be reported as a percentage of the measurement, which is known as **relative uncertainty**. To calculate the relative uncertainty, apply this formula:

$$\text{relative uncertainty} = \pm\left(\frac{\text{absolute uncertainty}}{\text{measurement}}\right)\times 100$$

Worked example 1.5.3

CALCULATING RELATIVE UNCERTAINTY FOR INSTRUMENTAL ERROR

A student observing the coverage of lichen on a tree trunk measured 43 cm^2 of lichen coverage in a 10 cm × 10 cm quadrat with 1 cm^2 measuring squares (increments). Calculate the relative uncertainty.	
Thinking	**Working**
Write down the formula for relative uncertainty.	$\text{relative uncertainty} = \pm\left(\frac{\text{absolute uncertainty}}{\text{measurement}}\right)\times 100$
Insert the values then perform the calculation.	$\text{relative uncertainty} = \pm\left(\frac{0.5}{43}\right)\times 100$ $= \pm 1.16$ $= 1\%$
Combine the measurement and uncertainty.	43 ± 1% cm^2

➤ Try yourself 1.5.3

CALCULATING RELATIVE UNCERTAINTY FOR INSTRUMENTAL ERROR

A student observing species diversity in a 10 m × 10 m dry sclerophyll forest measured the soil pH at 6.2 using an old digital sensor. Calculate the relative uncertainty.

Significant figures

The measurement or observation during experimentation, which is dependent on the precision of the instrument, determines the number of significant figures and decimal places you record in the data.

Significant figures are the numbers that convey meaning and precision. Using either a greater or smaller number of significant figures is misleading. Significant figures are the exact digits observed when recording the results of an observation or experiment according to the precision of the instrument. For example, if the volume of O_2 gas produced and measured from a plant photosynthesising during a six-hour experiment was 6.0 mL, because exactly 6.0 mL was observed, then the measurement is 6.0 mL with two significant figures, not 6 mL.

> It is important to record the exact numbers measured during an experiment or practical. This determines the significant figures, and later the accuracy and precision of the analysis. If you observe a zero, record it because it is the exact amount measured. This includes all zeros before and after decimal places.

SKILLBUILDER

Determining the significant figures of a measurement from an instrument

The recorded values, including significant figures, are determined by the precision (increments) of the instrument. The plastic pipette in Figure 1.5.3 measures up to 5 mL and has 1 mL increments. As the instrument measures in whole millilitre increments, the recorded value must be to a whole millilitre.

If you measured 2 mL with the pipette in Figure 1.5.3, then you should measure 2 mL, not 2.0 mL. This is because the measurement was not precise to one-tenth (0.1) of a millilitre. Therefore, using this instrument, the value is measured and recorded to one significant figure.

To determine how many significant figures there are in a measurement, follow these rules.

1. All non-zero digits are significant.
2. All zeros between non-zero digits are significant.
3. Trailing zeros (either decimal or not), if measured, are significant.
4. Leading zeros are not significant (in numbers with decimals, they only inform where the decimal is placed).

Table 1.5.3 shows some examples of measurements and how many significant figures they have. Review the examples to learn more about significant figures.

FIGURE 1.5.3 This 5 mL plastic pipette has 1 mL increments.

TABLE 1.5.3 Measurements and significant figures

Measurement	Number of significant figures
15	2
3.5	2
3.50	3
0.037	2
1401	4

Calculations with uncertainties

There are a few rules when conducting calculations involving numbers (or measurements) with uncertainties. The correct uncertainty, which indicates accuracy, needs to be reported once all the calculations are done. Table 1.5.4 outlines the rules for calculations involving data with uncertainty.

Keep in mind that the rules for significant figures apply to all numerical reporting, including the uncertainties.

TABLE 1.5.4 Rules for calculations involving uncertainty

Calculation	Rule	Examples
addition and subtraction	Add the uncertainties.	$5.4 \pm 0.05 + 3.2 \pm 0.05 = 8.6 \pm 0.1$ $5.4 \pm 0.05 + 3.2 \pm 0.1 = 8.6 \pm 0.15$ $5.4 \pm 0.05 - 3.2 \pm 0.05 = 2.2 \pm 0.1$ $5.4 \pm 0.05 - 3.2 \pm 0.1 = 2.2 \pm 0.15$
multiplication and division	Add the relative uncertainties.	$3.0 \pm 0.05 \times 1.5 \pm 0.05$ $= 3.0 \pm 1.7\% \times 1.5 \pm 3.3\%$ $= 4.5 \pm 5.0\%$ $3.0 \pm 0.05 \div 1.5 \pm 0.05$ $= 3.0 \pm 1.7\% \div 1.5 \pm 3.3\%$ $= 2.0 \pm 5.0\%$
multiplying with a constant (absolute uncertainty)	Multiply the uncertainty.	$3.14 \times (4.1 \pm 0.05) = 12.9 \pm 0.16$
multiplying with a constant (relative uncertainty)	Maintain the uncertainty.	$3.14 \times (4.1 \pm 1.6\%) = 12.9 \pm 1.6\%$

Uncertainty due to nature

In nature, there are many sources of variation that make it impossible to measure the same value twice. Examples of variations in nature are shown in Figure 1.5.4. They include:

- genetic differences
  - variation in the genetic code
  - variation in the regulation of the code
- intracellular differences in molecular processes and capacity
- extracellular differences in the maintenance and relationships between cells, tissues, organs and systems
- differences in how organisms relate (intraspecies and interspecies relationships)
- various abiotic factors affecting biotic factors in habitats and ecosystems
- local, regional and global differences in ecological relationships.

Natural biological variations can be known as genetic variation, biological variation or natural variation. Genetic variation is often the cause of biological and natural variation (though not always). When reporting natural, biological or genetic variation, it is best to choose one term and use it consistently.

The difficulty with uncertainty due to nature is that the true value can never be known or does not exist for a population. Most biological experiments are open-system and therefore influenced by numerous variables. Also, most biological experiments measure non-invasively or indirectly, making it difficult to achieve accuracy. Due to typically measuring a range of values in biology, acknowledging and recording accuracy and precision is necessary for analysis and interpreting the results. Statistical analysis greatly helps analysing and interpreting the data (Module 1.7).

FIGURE 1.5.4 (a) Close inspection of the bees reveals variation in the shades of colour and banding. (b) The same species of plant can be affected by different pH values in the soil, displaying variation within a habitat. (c) Genetic variation within a family. (d) Genetic variation within plants shown by wrinkled peas (left) and round peas (right).

1.5 Review

SUMMARY

- Accuracy is the ability to obtain the correct measurement. To obtain accurate results, you must minimise systematic errors.
- Precision is the ability to consistently obtain the same measurement. To obtain precise results, you must minimise random errors.
- Uncertainty is a range of measurements from the experiment in which the true value is thought to be.
- Error is the difference between a measurement and the true value. This is not an indication of human error or maintaining scientific validity.
- Uncertainty and error values are presented as a range (e.g. ±0.25).
- A simple way to calculate uncertainty is:

$$\text{uncertainty} = \pm\frac{\text{maximum value} - \text{minimum value}}{2}$$

- A simple way to calculate the absolute uncertainty is:

$$\text{absolute uncertainty} = \pm\frac{\text{smallest increment}}{2}$$

- A simple way to calculate the relative uncertainty is:

$$\text{relative uncertainty} = \pm\left(\frac{\text{absolute uncertainty}}{\text{measurement}}\right)\times 100$$

- The rules for calculations involving uncertainty are:

Calculation	Rule
Addition and subtraction	Add the uncertainties
Multiplication and division	Add the relative uncertainties
Multiplying with a constant (absolute uncertainty)	Multiple the uncertainty
Multiplying with a constant (relative uncertainty)	Maintain the uncertainty

- Significant figures are the numbers that convey meaning and precision. The number of significant figures used depends on the scale of the instrument.
- The rules governing significant figures are as follows.
  - All non-zero digits are significant.
  - All zeros between non-zero digits are significant.
  - Trailing zeros (either decimal or not), if measured, are significant.
  - Leading zeros are not significant (in numbers with decimals, they only inform where the decimal is placed).

KEY QUESTIONS

Retrieval

1 Write the symbol for uncertainty.

2 Define systematic error.

3 Define random error.

Comprehension

4 Explain the difference between accuracy and precision.

5 Determine the instrumental error in this instrument.

6 Write the temperature measured by the thermometer, including the instrumental error.

7 The pipette shown at right is a 10 mL glass pipette with 0.1 mL increments. If exactly 2 mL was measured using this pipette, write the value with the appropriate number of significant figures.

Analysis

8 Calculate the average and uncertainty of these two sets of data.

a Dataset A: 11.4, 10.9, 11.8, 10.6, 11.5, 11.1

b Dataset B: 25.3, 27.4, 22.0, 26.1, 28.4, 23.1

9 Identify the following scenarios as examples of random or systematic error.

a You weigh the mass of a powder three times, using the same scales and get three different readings.

b The tape measure you are using to measure distance has been stretched.

c When electronic scales are zeroed, they read 0.05 g.

1.6 Tables and graphing

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- create tables to record and organise data
- present data from tables in graphs
- distinguish between types of graphs
- select appropriate graphs to represent data
- represent missing data in graphs
- explain what outliers are and know how to represent them in graphs.

PRESENTING DATA IN TABLES

Tables record number values and allow you to organise your data. In general, tables provide more detailed data than graphs, but it is easier to observe trends and patterns in data in graph form than in table form.

Presenting raw data in tables

Tables organise data into rows and columns. They can vary in complexity according to the nature of your data. Tables can be used to organise raw data and processed data (Module 1.10), or to summarise results.

The simplest form of a table is a two-column chart. The first column should contain the **independent variable** (the one you control) and the second column should contain the **dependent variable** (the one that may change in response to a change in the independent variable).

As you can see in Figure 1.6.1, tables should have the following features:

- a descriptive title
- column headings (including the units)
- aligned figures (align the decimal points)
- independent variables in the left column(s)
- dependent variables in the right columns.

Table 1: The effect of pH on plant growth

pH of water	Plant number	Plant mass (g) for each day of the trial											
		Trial 1						Trial 2					
		0	2	4	5	6	10	0	2	4	6	8	10
5.0	1												
	2												
	3												
	4												
	Average												
7.0	1												
	2												
	3												
	4												
	Average												
9.0	1												
	2												
	3												
	4												
	Average												

Accurate, descriptive title.

Independent variable in the left column.

Dependent variable identifies the data set and shows the units of measurement.

Space left to calculate averages.

Space for trials—in this case two repeat trials were conducted.

Space for recording the dependent variable values.

Rows show the different treatments—the range of values for the independent variable.

Each row shows a different organism (plant)—in this case four replicates at each pH level.

FIGURE 1.6.1 Examples of features to be included in tables.

TABLE 1.6.1 Effect of temperature on mean transpiration rate, including uncertainty

Temperature (°C)	Mean transpiration rate ($mL\,g^{-1}\,h^{-1}$)
15	0.038 ± 0.002
25	0.043 ± 0.001
35	0.059 ± 0.001
45	0.074 ± 0.0015

Presenting processed data in tables

Table 1.6.1 shows the relationship between temperature and mean transpiration rate (processed data). The uncertainty is also displayed, which will be a requirement for assessments. As required, the column headings include the units.

PRESENTING DATA IN GRAPHS

Graphs are used to display relationships, trends and patterns between two variables with one proposed to be dependent on the other, as shown in Figure 1.6.2.

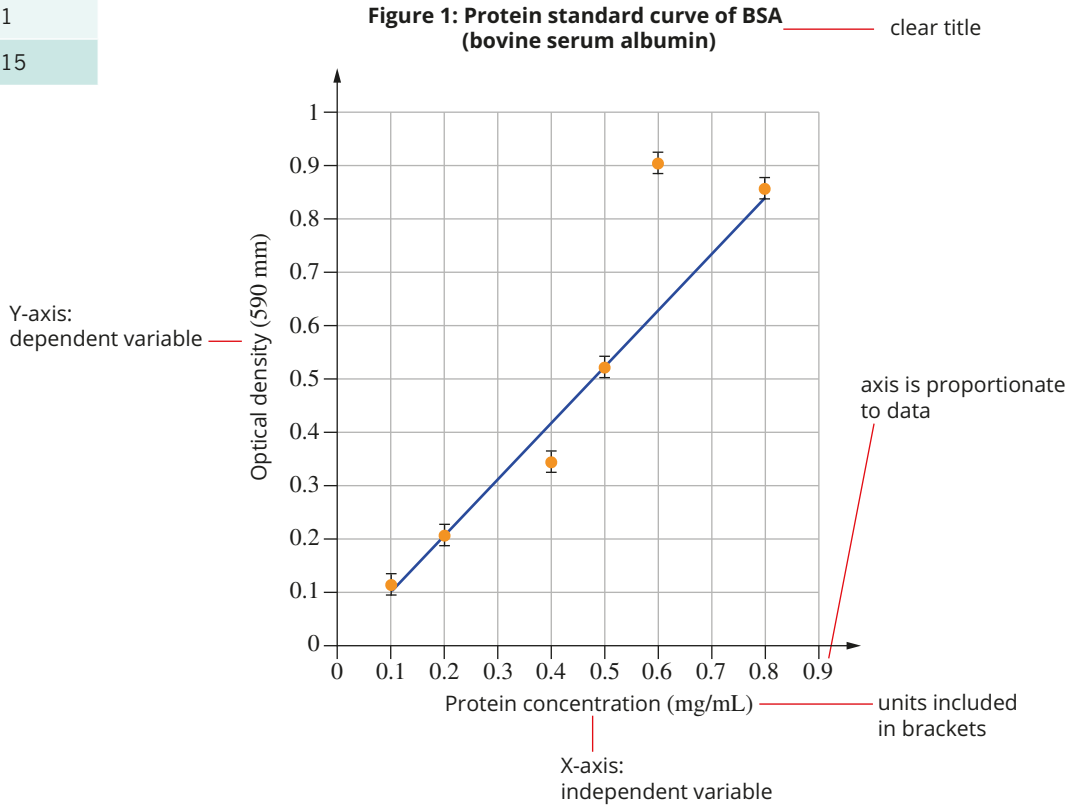


FIGURE 1.6.2 Examples of features to be included in graphs.

There are several types of graphs, including line graphs, bar graphs, scatterplots and pie charts. The best one to use will depend on the nature of the data. General rules to follow when making a graph include the following.

- Keep the graph simple and uncluttered.
- Use a descriptive title that includes the independent and dependent variable.
- Represent the independent variable on the *x*-axis and the dependent variable on the *y*-axis.
- Start each axis at zero.
- Match the length of the axes to the data.
- Clearly label axes with both the variable and the unit in which it is measured.
- Use small symbols such as circles or squares for data points.
- Use different symbols for different datasets.
- Display discrete and continuous data appropriately.

Scatterplots and line graphs

Scatterplots are appropriate and often best for continuous and discrete data, large volumes of data and raw data. Qualitative and quantitative data (both discrete and continuous) can be displayed using scatterplots. They are used to show the relationship between two variables when one variable is dependent on the other.

The data is plotted on the graph as a series of points. Each point should be drawn in pencil as a small circle or cross. Alternatively, you can use a computer program to generate your graphs.

A **line graph** is a good way of representing continuous quantitative data. In a line graph, the values are plotted as a series of points on the graph. A line is drawn from each point to the next, as shown in Figure 1.6.3. This line shows the change in data from one point to the next of the same sample or subject (hence continuous data), but does not predict the value between the plotted data.

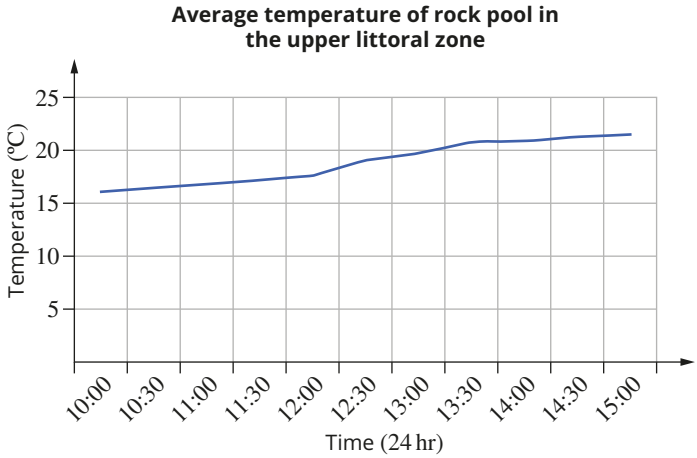


FIGURE 1.6.3 A line graph showing the average temperature (°C) of a rock pool in the upper littoral zone throughout the day, with lines ruled from each sample point to the next.

Bar and column graphs

Bar and column graphs are used to show categories (categorical data) and discrete (discontinuous) data (Module 1.8).

- A **column graph** shows the value of the dependent variable by the height of the column. The categories are labelled across the *x*-axis.
- A **bar graph** shows the value of the dependent variable by the length of the horizontal bar. The categories are labelled up the *y*-axis.

The bars or columns are always the same width and the same distance apart. Figure 1.6.4 is an example of a column graph displaying the discrete quantitative data of the number of base pairs and number of genes on each human chromosome. Note that two different vertical axes are used for the different datasets, which have very different scales.

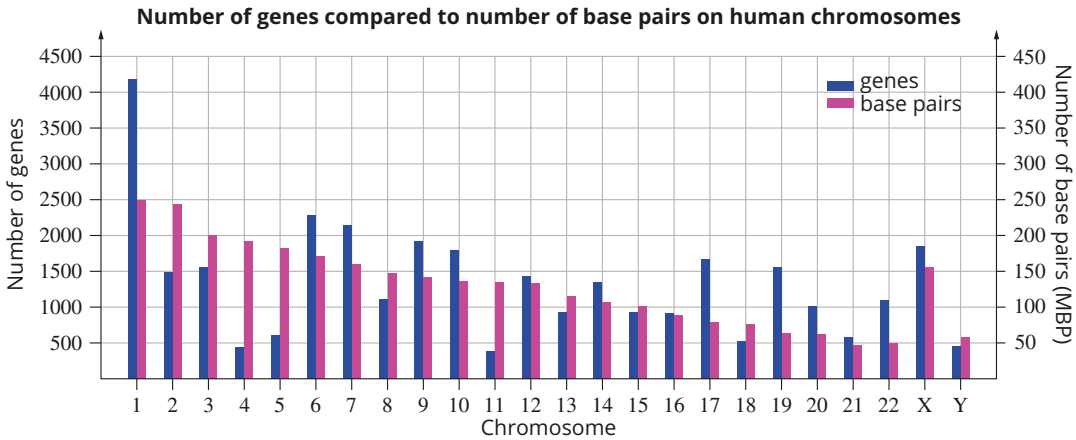


FIGURE 1.6.4 A column graph comparing the number of genes (blue, left *y*-axis) and the number of base pairs (pink, right *y*-axis) on human chromosomes.

When the labels of the variables are long, horizontal bar graphs can be used. Bar graphs are also used when the data ranges are variable and overlapping, such as genome sizes, as shown in Figure 1.6.5.

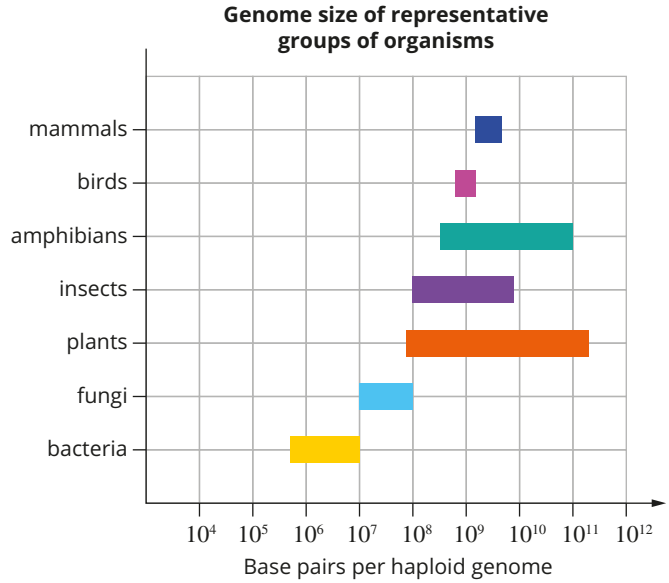


FIGURE 1.6.5 A horizontal bar graph comparing genome size of representative organisms from the different kingdoms of life.

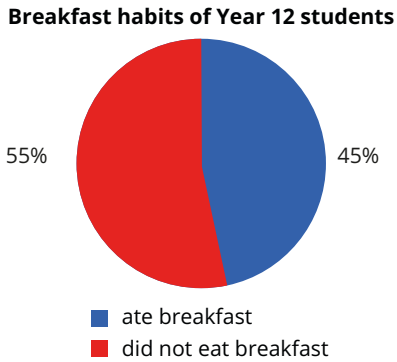


FIGURE 1.6.6 A pie chart presenting data on the breakfast habits of students.

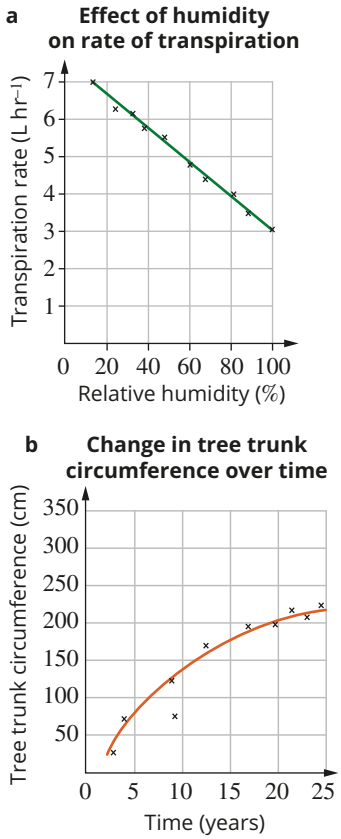


FIGURE 1.6.7 Scatterplots showing (a) straight and (b) curved trend lines.

Pie charts

A **pie chart** is a way of presenting qualitative or discrete quantitative data (Module 1.9). It shows each category of data as a proportion of the total data. The chart is a circle divided into sections according to the proportions of each category, like slices of a pie (Figure 1.6.6). Each category is coloured or shaded differently so that it can be clearly distinguished from the other categories. Pie charts are only recommended when there are few categories.

A circle is equal to 360°. To draw a pie chart, you must determine how many degrees are needed for each category. This can be done as follows:

1. Add the amounts in each category to find the total.
2. Divide 360° by the total (this will tell you how many degrees of the circle one value is worth).
3. Multiply the answer by the amount in the first category. Your answer will be in degrees that can then be marked for the first category using a protractor on the circle.
4. Repeat for each category.

Graph features

Some features can be used in graphs to show more detail. Features that can be added to graphs include trend lines, anomalies, outliers and uncertainty. Additional features should only be used when they add more understanding to the information. Also, all information in a graph should be explained in the analysis, interpretation or evaluation section of a report; therefore, only display features you can explain.

Trend lines

A single straight or curved line that best fits the data on a graph, as shown in Figure 1.6.7, is a **trend line**. It is used to show the overall trend in the data and can be used to predict values between the data points. The trend line usually does not pass through every data point but there must be an equal number of data points above and below the trend line. More accurate trend lines can be drawn using programs that calculate the line mathematically from the data (Module 1.7).

Anomalies and outliers

When a measured result or data point does not fit the trend or relationship, it is called an **anomaly**. Anomalies are often caused by random errors (Module 1.5) or atypical biological variation. When an anomaly can be determined to be statistically different to the other data it is called an **outlier**. Outliers are data that sit outside the statistical range or accepted values according to mathematical analysis (Module 1.7). They can only be determined by statistical calculations, not using a trend line. If you have an outlier in your results, you include it in your graph but ignore it when drawing the trend line (Figure 1.6.8). The analysis of anomalies and outliers will be discussed in Module 1.10.

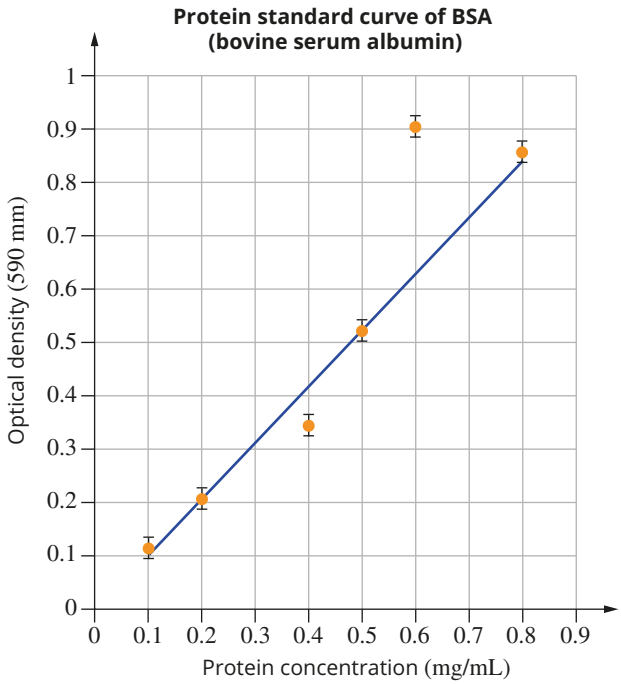


FIGURE 1.6.8 A line graph showing an outlier, which has been ignored when adding the line of best fit.

Missing data

When you have missing data, leave a gap for it, as shown in Figure 1.6.9. Ensure that the axes are complete (do not skip values) and do not join the data points that have missing data points between them.

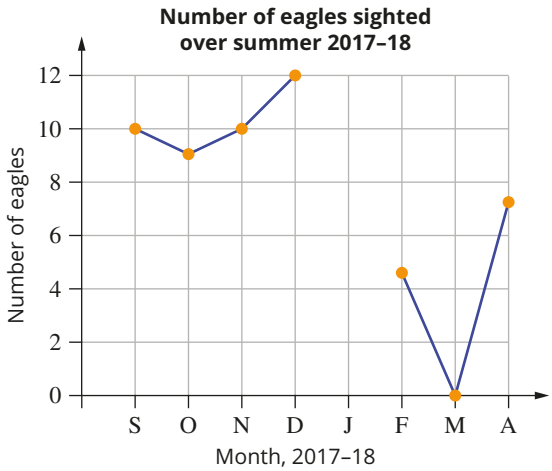


FIGURE 1.6.9 A line graph with missing data. This accurately represents the measurements taken during the methodology.

Distorting the truth

Poorly constructed graphs can distort the truth and not display the true trend, pattern or relationship in the data. For example, in Figure 1.6.10 you can see two graphs that show the same data—the test results of two groups of students. One group of students did not eat breakfast before doing the test, and scored an average of 42 marks out of 50. The other group of students did eat breakfast and scored an average of 48 marks out of 50. Graph 2 distorts the difference in marks between the two groups by using a scale of only 40 to 50 marks on the *y*-axis.

It is important to make sure the graphs you create do not distort your data. You should also be wary of distorted data when interpreting graphs in other publications.

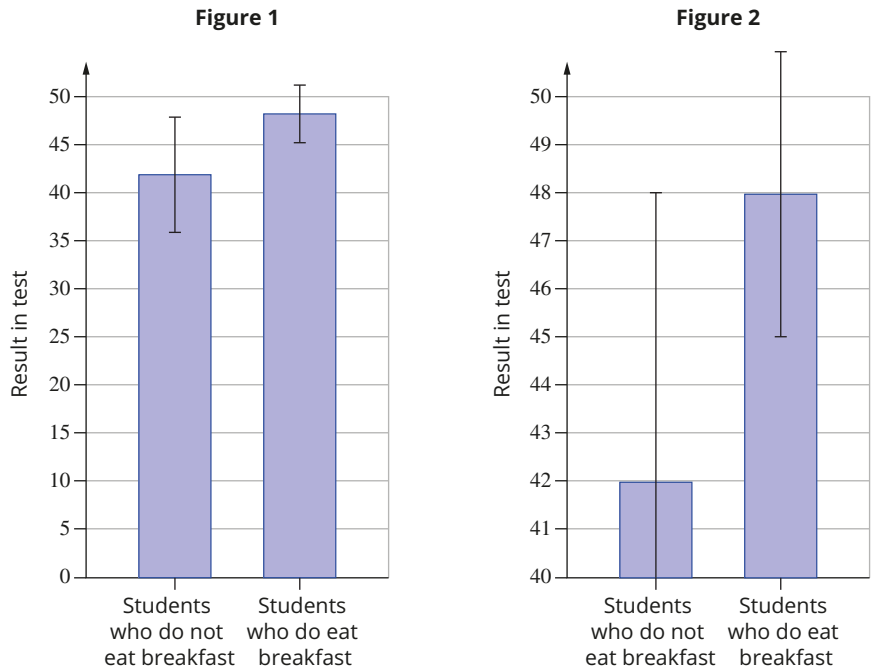


FIGURE 1.6.10 Figure 1 shows the difference between two sets of test results on the *y*-axis. Figure 2 uses only a narrow range of marks on the *y*-axis, which distorts the difference and makes it appear larger than it really is.

1.6 Review

SUMMARY

- Tables and graphs are used to represent scientific data.
- In general, tables provide more detailed data than graphs, but it is easier to observe trends and patterns in data in graph form than in table form.
- Tables organise data into rows and columns, and can vary in complexity according to the nature of your data.
- Tables should have:
  - a descriptive title
  - column headings (including the units)
  - aligned figures (align the decimal points)
  - the independent variables in the left column
  - the dependent variables in the right column.
- Graphs are used to display relationships, trends and patterns between two variables, with one proposed to be dependent on the other.
- There are several types of graphs, including line graphs, bar graphs and pie charts. The best graph to use will depend on the nature of the data.
- Scatterplots are commonly used to display data in the form of a graph and can be used to plot raw or processed data.
- A trend line is used to show the overall trend in the data and can be used to predict values between the data points.
- A line graph is a good way of representing continuous quantitative data.
- Bar and column graphs are used to show categories and discrete (discontinuous) data.
  - A column graph shows the value of the dependent variable by the height of the column. The categories are labelled across the *x*-axis.
  - A bar graph shows the value of the dependent variable by the length of the horizontal bar. The categories are labelled up the *y*-axis.
- A pie chart is a way of presenting qualitative or discrete quantitative data.
- Missing data is represented in graphs by leaving a gap.
- An outlier is a data point that does not fit the trend, and may be caused by an error.

KEY QUESTIONS

Retrieval

1 State the purpose of a trend line.

2 List the type of data that can be displayed in a scatterplot.

Comprehension

3 Describe an outlier and how it affects the mean.

Analysis

4 Contrast anomalies and outliers.

5 Identify when it is more appropriate to use a graph showing a trend line rather than a ruled line graph joining the line from point to point.

6 An ecological study observed the competition between an introduced invasion of a grass (*Microstegium vimineum*) and natural flora in a plot 60 m × 60 m. The results for the biomass of *Microstegium vimineum* is shown below.

a Draw a graph to show the data over time.

Biomass of *Microstegium vimineum*

Year	1	2	3	4	5	6	7	8
Experiment plot biomass ($g\,m^{-2}$)	380 ± 30	295 ± 35	385 ± 65	475 ± 55	200 ± 25	75 ± 15	15 ± 5	0
Control plot biomass ($g\,m^{-2}$)	0	0	0	10 ± 5	15 ± 7	0	0	0

b Identify the element of the graph that makes the drawing of the Year 4 and 5 uncertainty of the control plot biomass difficult.

1.7 Statistics

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- explain and calculate the following features of datasets: range, mean, median and mode
- explain the statistical significance of standard deviation
- understand that various statistical methods are used to analyse datasets, including confidence intervals, student *t*-tests and *P* values.

Some difficulties with data obtained in the biological sciences include:

- most measurements are indirect rather than direct
- experiments are more often open-system rather than closed system
- natural variation always exists.

The true value of measurements is rarely obtained, so results must display limitations and uncertainty (Module 1.5), and errors related to reliability and validity (Modules 1.8 and 1.9). Performing statistical analysis will indicate the quality of the measurements and help interpretation.

This module develops understanding of statistics that will help with completing all syllabus assessments. Many statistical calculations have been described by the biology syllabus support material.

This module provides guidance on a number of different statistical calculations. Some of these calculations will be useful for the student experiment, while others will markedly help understanding data used for the data test and research investigation.

Completing statistical calculations can sometimes involve complex formulas and be time-consuming. As such it is easy to make an error when performing these calculations. Using software (e.g. Microsoft Excel) is a more efficient option for most statistical calculations. If the formula has not been provided here in this section, then software has been used to complete the calculation.

Important note: Interpreting statistics and the values produced is only valid within the sample tested or experiment conducted. Applying the statistics to the rest of the world is generally invalid. It is important to understand that many statistical calculations assume that the tested sample represents the world's population for the chosen organism or variable. Also, a normal distribution is assumed, including a range of results from extreme, yet rare values to common, high frequency values. For example, the values calculated when using standard deviation, standard error, confidence interval, student *t*-test and *P* value all assume a normal distribution and that the calculated values refer to the entire world's population, or the likelihood it would occur in the entire world's population. However, since the statistics are dependent on the measured results from the chosen sample, the estimated normal distribution is not true. The interpretation of the statistical values is limited and only valid for the chosen sample, within the scope of the experiment and research question (or hypothesis), the associated controlled and observed variables, and the biases within the experimentation.

Medical and pharmaceutical research undergoes many rounds of trials, each time increasing the sample size from hundreds to tens of thousands. More than 90% of developed drugs and medicines do not make it through the trials because, as the sample size increases, the early promising results according to statistical calculations fade into insignificance as a more accurate normal distribution is obtained.

RANGE

The range is the difference between the highest and lowest values in a dataset. Table 1.7.1 shows the canopy percentage coverage (shade) of the forest floor for five different 10 m × 10 m quadrats in a temperate open forest.

TABLE 1.7.1 Percentage canopy coverage of forest floor

	Quadrat					Mean	Range
	1	2	3	4	5		
canopy coverage (%)	80	95	85	85	75	84	95 – 75 = 20

To determine the range for the data in Table 1.7.1, you subtract the smallest value from the largest value. Interpreting the range is quite simple. It illustrates the amount of variation in the data. A large range can be interpreted in a variety of ways. It can suggest further analysis is required to search for errors, anomalies or outliers (if a large range was not expected); it can suggest further analysis to investigate if natural variation is responsible; or it can demonstrate poor methodology design or sample choice. A small range may indicate excellent control of variables and a highly reliable methodology, or it may indicate insufficient sampling.

MEASURES OF CENTRAL TENDENCY

Measures of central tendency are single values that allow you to describe the central position in a set of data. The mean, median and mode are all measures of central tendency.

Consider the dataset: 3, 5, 7, 8, 8, 8, 10.

- The mean, $\bar{x}$, is the average of a dataset. It is calculated by dividing the sum of the values by the number of values:

$$\bar{x} = \frac{\Sigma x_1 + x_2 + x_3 + \ldots}{n}$$

where

Σ = sum of $x_1 + x_2 + x_3$ = measurement$_1$ + measurement$_2$ + measurement$_3$

n = total number of measurements.

In this case, the mean is $(3 + 5 + 7 + 8 + 8 + 8 + 10) \div 7 = 7$.

- The **median** is the middle value in an ordered list of values, which in this case is the fourth value, 8.
- The **mode** is the value that occurs most often in a list of values, which in this case is 8. This measure is particularly useful for describing qualitative or discrete data.

The appropriate measure of central tendency to use depends on the type of data you are working with (Table 1.7.2).

Graphically representing the data using central tendency provides a clear and succinct display of results. It is also less challenging to interpret trends, patterns and relationships using summarised values rather than the entirety of the raw data. Keep in mind that central tendency is not the true value as it is a model representation for numerous values. Table 1.7.3 is an example of two means (average) that have been processed from raw data. Graphically representing the means rather than the raw data will be more succinct with less clutter.

TABLE 1.7.2 When to use the different measures of central tendency

Type of data	Mean	Median	Mode
nominal (qualitative)	✓	×	×
ordinal (qualitative)	✓	✓	maybe
discrete or continuous (quantitative)	✓	✓	✓

TABLE 1.7.3 Measured circumference of mature flooded gum in open and closed temperate forest

Circumference of flooded gum in closed temperate forest (m)	Circumference of flooded gum in open temperate forest (m)
3.76	6.28
5.28	5.68
4.72	5.01
3.92	4.72
5.76	5.31
4.04	4.98
mean 4.58	mean 5.33

STANDARD DEVIATION

Standard deviation specifically estimates the spread of data in a population or set of values and its distance from the mean. It is a descriptive statistic, which can help describe the quality of the data.

The standard deviation calculation assumes that the data being used is from a set in which a normal curve (or normal distribution) exists. A normal distribution of data forms a 'bell' curve or a normal curve, as seen in Figure 1.7.1. A normal curve on a graph has:

- approximately 68.2% of all the data within one standard deviation (1σ) of the mean; that is, 34.1% above the mean and 34.1% below the mean
- approximately 27.2% of all the data between the first and second standard deviation (between 1σ and 2σ); that is, 13.6% above 1σ and 13.6% below 1σ
- approximately 4.2% of all data between the second and third standard deviation (between 2σ and 3σ); that is, 2.1% above 2σ and 2.1% below 2σ
- the final 0.2% above and below 3σ, 0.1% on either side.

The standard deviation will indicate the size of variation in a normal curve, that is, the distance from the mean where 68.2% of results can be found, the distance from the mean for the next 27.2% of results, and so on. The larger the standard deviation, the greater the distance from the mean. Table 1.7.4 (page e44) illustrates this displaying the height of all the female students in two Year 12 biology classes.

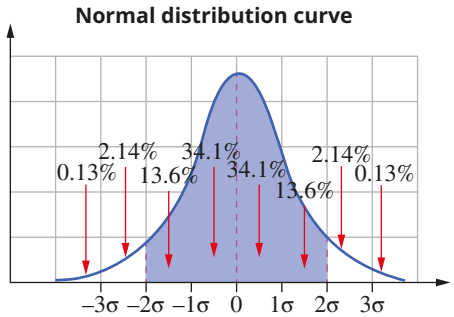


FIGURE 1.7.1 Normal distribution curve showing how far each standard deviation (σ) is from the mean (0).

TABLE 1.7.4 Height of female students in two Year 12 biology classes

Sample	Height (cm)	
	Class A	Class B
student 1	164.5	162.5
student 2	156.7	166.1
student 3	172.2	182.7
student 4	180.6	186.0
student 5	163.4	163.3
student 6	158.8	149.8
student 7	153.9	147.2
student 8	150.7	155.7
student 9	160.4	144.9
student 10	156.8	153.5
mean	161.8	161.2
standard deviation	8.9	14.1

The mean height in both classes is equivalent; however, the standard deviation is different. For class A, the standard deviation has calculated that 68.2% of all Year 12 female students will be within 8.9 cm of the mean. This is 1σ. For class A, 2σ is 2 × 8.9 cm, which is 17.8 cm from the mean (above and below). For class B, one standard deviation (1σ) is 14.1 cm from the mean. The normal curve for class A is shown in Figure 1.7.2.

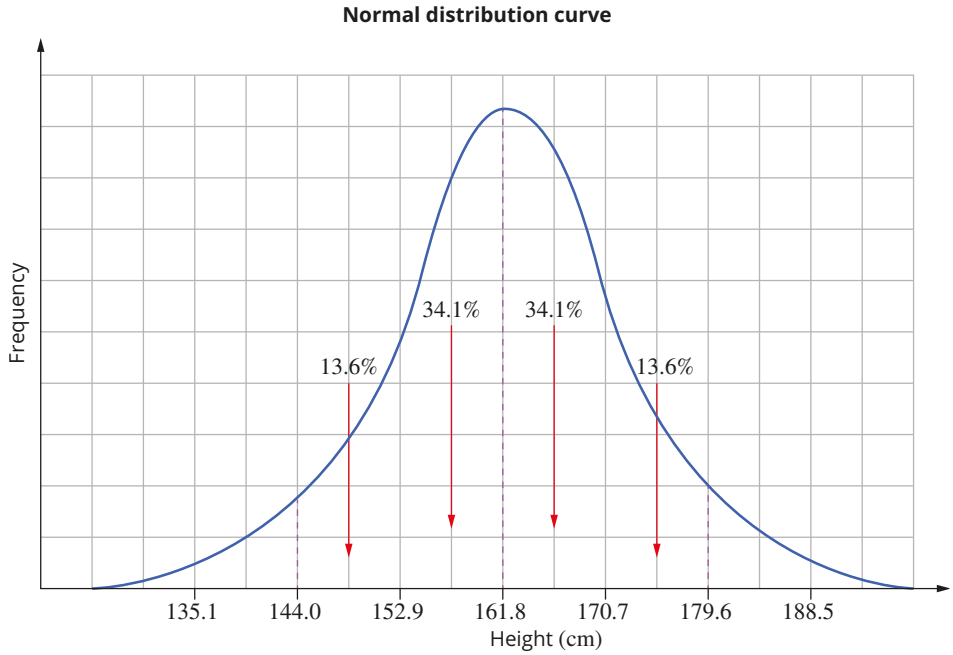


FIGURE 1.7.2 Normal curve demonstrating the standard deviation distribution of students in class A. The mean height is 161.8 cm, and each increment above and below the mean on the *x*-axis is one standard deviation.

Being a descriptive statistic, the standard deviation can be used to analyse the validity of the method or analyse the sample. For the data in Table 1.7.4, the means are equivalent; however, the standard deviation is different. The standard deviation describes the variation in the data and class B has more variation than class A. This is either due to the methodology or the samples, so either one or none of the samples represent the true population.

A descriptive analysis from this data could say that student 4 in class A (180.6 cm) is more than 2σ away from the mean, and is estimated to be in the top 3% of the Year 12 female student population for height. This data contradicts the results from class B. Another interpretation can be to assume that anything equal to or greater than 3σ is an anomaly or outlier.

For this dataset, the standard deviation clearly shows a difference in variation for the two samples (class A and class B). An analysis of the standard deviation would then be used to describe the samples and their differences.

STANDARD ERROR

Because science includes uncertainty and errors, the measured mean will always deviate from the true population mean (or accepted population mean). Many factors can cause this, so one of the most valuable measures to determine the accuracy of the sample (dataset) is standard error. Standard error estimates the accuracy with which a sample represents a population. The deviation from the true or accepted mean of a population is the standard error.

The calculation for standard error of the mean can estimate how close the measured mean is to the accepted or expected population mean. Lower values for standard error indicate the measured mean may be close to the true or accepted population mean. Larger values for standard error indicate the measured mean may be further away from the true or accepted population mean.

The formula for standard error is:

$$SE = \frac{\sigma}{\sqrt{n}}$$

where

SE = standard error

σ = standard deviation

n = number of samples.

A larger standard deviation will affect the standard error and result in a larger value for the standard error. The smaller the number of samples, the larger the value for the standard error will be. The larger the sample size, the more precise the standard error estimate (value) will be.

The statistical results in Table 1.7.5 are based on the data in Table 1.7.4. The standard error has been calculated and it can be seen that the value for class B is larger than class A. In a summary of the results, it would be said that the standard error estimates the true or accepted population mean within ±4.5 cm of 161.2 cm for class B. The accepted population mean is within ±2.8 cm of 161.8 cm for class A.

An interpretation of these results would explain that the larger standard error of class B means its samples and resultant mean are less reliable. In analysing the formula for standard error, only the standard deviation and sample size can affect the value of standard error. As class A and B both had 10 samples (Table 1.7.4) and both calculated an equivalent mean, the larger standard deviation in class B must have caused the result. We can conclude that the standard error is larger in class B due the larger variations in the sample. Its chosen sample population is less representative of the true population and has caused the results to produce a wider estimation as to where the accepted population mean could be found.

Standard error is one of the most valuable measures to determine the accuracy of a sample (dataset).

TABLE 1.7.5 Statistical results for measuring the height of female students in two Year 12 biology classes

	Class A	Class B
Number of samples	10	10
Mean (cm)	161.8	161.2
Standard deviation (cm)	8.9	14.1
Standard error (cm)	2.8	4.5

CONFIDENCE INTERVAL

The **confidence interval** estimates the reliability of an experimental method. The calculation uses the variations in the measured values to infer the level of uncertainty related to the method and the overall measurements of data. Because scientific experimentation always includes uncertainty and error (Module 1.5) it is helpful to estimate the overall uncertainty of an experiment.

The confidence interval is not a direct measure of uncertainty or error in measurements and should never be used to replace them. They must always be reported. The confidence interval assumes there are other factors that have also influenced the quality of the method, some of which are unknown to the scientists conducting the experiment, for example, selection bias or human influence.

The confidence interval produces a range in which, if the experiment was conducted again (under the same conditions), an equivalent value (e.g. mean) would be obtained. This is done by using the variations (differences) in the measured values in the calculation. It therefore describes reliability and uncertainty (Table 1.7.6).

TABLE 1.7.6 Examples of confidence intervals (CI) and their interpretation

Mean (cm)	Predetermined confidence level	CI (cm)	Results (cm)	CI range (cm)	Interpretation
175.6	98%	6.6	175.6 ± 6.6 cm	169–182.2 cm	If this experiment were conducted again, there is a 98% probability that the mean obtained will be determined ±6.6 cm from 175.6 cm (between 169 cm and 182.2 cm).
175.6	95%	5.5	175.6 ± 5.5 cm	170.1–181.1 cm	If this experiment were conducted again, there is a 95% probability that the mean obtained will be determined ±5.5 cm from 175.6 cm.
175.6	90%	4.6	175.6 ± 4.6 cm	171–180.2 cm	If this experiment were conducted again, there is a 90% probability that the mean obtained will be determined ±4.6 cm from 175.6 cm.

The calculation requires the experimenter to first determine a value that is used in the calculation—the confidence level (or level of confidence). For example, the experimenter must choose a value of 95% or 90%, or any other value, and insert it into the calculation.

As can be seen in Table 1.7.6 (page e45), the confidence interval calculation determines the confidence interval range (CI range). This range indicates (describes) the level of uncertainty in the results. The size of the range is due to the predetermined confidence level that was inserted into the calculation (e.g. 98% or 95%). The larger the confidence level (98% compared to 90%), the larger the CI range of uncertainty. The confidence interval calculation can never use a 100% confidence level. Commonly, a 95% confidence level is used when calculating the confidence interval. Studies with large sample sizes often use higher confidence levels and sometimes a 90% confidence level may be used with small sample sizes. Also, ground-breaking studies or studies with expected large variations in the data will use lower confidence levels.

An analysis of the confidence interval can describe the quality of the methodology. The smaller the CI range, the more controlled and reliable the experiment. The larger the CI range, the less reliable it is.

Worked example 1.7.1

CALCULATING THE CONFIDENCE INTERVAL TO COMPARE THE UNCERTAINTY FOR TWO GROUPS

A Year 12 biology class was performing a mandatory practical, using the process of stratified sampling to collect and analyse primary biotic and abiotic field data to classify an ecosystem during a rocky shore field trip. One set of primary biotic data the students had to collect included the size of red waratah anemones to determine their mean size. Group 1's results were 3.8, 4.2, 3.6, 3.7 and 2.5 cm. Group 2's results were 3.8, 4.2, 3.6, 3.7, 2.5, 3.4, 4.0, 3.9, 3.9 and 3.8 cm. Calculate the confidence intervals for each group's data and evaluate the quality of their method.

Thinking	Working
Record the raw data from the practical and place into Microsoft Excel.	

Sample	Group 1 (cm)	Group 2 (cm)
1	3.8	3.8
2	4.2	4.2
3	3.6	3.6
4	3.7	3.7
5	2.5	2.5
6	–	3.4
7	–	4.0
8	–	3.9
9	–	3.9
10	–	3.8

Calculate the mean using Microsoft Excel's 'insert function'.

Sample	Group 1 (cm)	Group 2 (cm)
1	3.8	3.8
2	4.2	4.2
3	3.6	3.6
4	3.7	3.7
5	2.5	2.5
6	–	3.4
7	–	4.0
8	–	3.9
9	–	3.9
10	–	3.8
Mean	3.6	3.7

Summarise the results.

The mean size of red waratah anemones in Group 1's and 2's samples were 3.6 cm and 3.7 cm respectively.

Calculate the confidence interval after calculating the standard deviation (as this is required for the confidence interval calculation). Then choose the CONFIDENCE.NORM from the Microsoft Excel 'insert formula' function. Type '0.1' in the alpha input (because we are choosing a 90% confidence level, there is a 10% residual and this becomes the alpha, written as 0.1). Insert the cell location for the standard deviation, then type in the number of samples.	
Summarise the results of the confidence interval.	Group 1 has a 90% probability of reproducing their red waratah mean within 0.5 cm, and Group 2 has a 90% probability of reproducing their red waratah mean within 0.2 cm. Group 2's results have a smaller range of uncertainty and therefore are more reliable.

Sample	Group 1 (cm)	Group 2 (cm)
1	3.8	3.8
2	4.2	4.2
3	3.6	3.6
4	3.7	3.7
5	2.5	2.5
6	–	3.4
7	–	4.0
8	–	3.9
9	–	3.9
10	–	3.8
Mean	3.6	3.7
CI (90%)	0.5	0.2

➤ Try yourself 1.7.1

CALCULATING THE CONFIDENCE INTERVAL TO COMPARE THE UNCERTAINTY FOR TWO GROUPS

On the same Year 12 biology class field trip and performing the same mandatory practical, student groups 3 and 4 sampled different quadrats with the following results. Group 3: 3.3, 3.3, 4.5, 2.7 and 3.1 cm. Group 4: 3.3, 3.3, 4.5, 2.7, 3.1, 3.8, 2.9, 3.7, 4.0 and 3.5 cm. Calculate the confidence intervals for each group's data and evaluate the quality of their method.

STUDENT *t*-TEST

The **student *t*-test** (also known as the *t*-test) compares two means and determines if there is a significant difference between them. It is an inferential statistic, meaning it infers if there is a significant similarity or difference between the measured results and the estimated true value of the world's population. It can produce a conclusion to address a hypothesis.

The *t*-test estimates the probability that the difference between two means is due to natural variation or chance when testing a **null hypothesis**. A null hypothesis supposes that no change or difference will be seen in the results when the independent variable is changed. Therefore, based on a null hypothesis, the *t*-test assumes the null hypothesis is true and that any variation between the sets is due to natural variation and not the independent variable.

The student *t*-test produces a *t*-value (between 0 and 1.00) which is interpreted as a percentage. A *t*-value of 0.04 is 4% and a *t*-value of 0.82 is 82%. A *t*-value below 0.05 is significant, and means that there is less than 5% chance that the difference between the means is due to natural variation. It is therefore probably due to the independent variable and the null hypothesis is rejected.

Anything higher than 0.05 is not significant. For example, a *t*-value of 0.62 means that there is a 62% probability that natural variation or chance caused the difference between the means (not the independent variable).

One of the unique features of the student *t*-test is that it can be used for small sample sizes, although it is limited to samples (datasets or sample groups) that are related.

There are two types of student *t*-tests: unpaired and paired (Table 1.7.7). An unpaired student *t*-test compares the means from two different samples. A paired student *t*-test compares the means of the same sample tested twice, the mean before and the mean after changing the independent variable.

TABLE 1.7.7 Examples of unpaired and paired student *t*-tests

Unpaired student *t*-test	Paired student *t*-test
Comparing the mean height of a group of pea plants grown in the sun and a group grown in the shade.	Comparing the mean rigour of tomato in potting mix for two weeks with the same group in soil with additional salt for two weeks.
Compare the mean chloroplast count of a sample of *Elodea* from one pond and another sample from another pond.	Comparing the mean chloroplast count of a sample of *Elodea* from fresh water in sunlight, and the same sample after being in the shade for two weeks.
Compare the Simpson's diversity index of the same type of bushland in two different locations.	Comparing the Simpson's diversity index of a bushland before and after back-burning.

Worked example 1.7.2

CALCULATING THE *t*-VALUE TO COMPARE MEAN NUMBERS

An investigation was conducted to see if canopy coverage has an effect on the number of plant species found growing on the forest floor (less than 2 m high). A null hypothesis was developed that there would be no difference in the number of plant species between canopy coverage <80% and >80%. Calculate the *t*-value to determine if the null hypothesis is supported or not.

Thinking | **Working**

Record the raw data from the experiment and place into Microsoft Excel.

Quadrat	Number of species (<80% canopy coverage)	Number of species (>80% canopy coverage)
A	7	11
B	5	12
C	11	9
D	8	11
E	8	13
F	10	8

Calculate the mean using Microsoft Excel's 'insert function'.

Quadrat	Number of species (<80% canopy coverage)	Number of species (>80% canopy coverage)
A	7	11
B	5	12
C	11	9
D	8	11
E	8	13
F	10	8
Mean	8	11

Summarise the results.

The mean number of plant species growing on the forest floor with <80% canopy coverage is 8, while the mean for >80% canopy coverage is 11. There is a difference in the means, but it is not known if it is significant. Both groups include variation. The number of plant species under <80% canopy coverage has a range of 6, while the range for >80% canopy coverage is 5.

Choose the *t*-test in Microsoft Excel's 'insert formula'. Select sample 1 (<80% results), then sample 2 (>80% results). Choose 2 tail, and then 2 again for the type of *t*-test.

Quadrat	Number of species (<80% canopy coverage)	Number of species (>80% canopy coverage)
A	7	11
B	5	12
C	11	9
D	8	11
E	8	13
F	10	8
Mean	8	11
t-value	0.06	

Summarise the results of the *t*-test.

There is a 6% chance that the difference in the means is due to natural variation. Even though the difference between the means is insignificant (greater than 0.05) there is a notable difference. The hypothesis is supported, and there is no significant difference in the number of species between canopy coverage greater and less than 80%, although the notable difference suggest further investigation is required.

➤ Try yourself 1.7.2

CALCULATING THE *t*-VALUE TO COMPARE MEAN NUMBERS

An investigation was conducted using stratified sampling to test if vertical strata exist in freshwater ponds associated with aquatic plants. One group of samples was collected from the base of the aquatic plants while the other group of samples was collected from the leafy foliage of the aquatic plants. A null hypothesis was developed that there would be no difference in the number of aquatic macroinvertebrate species between the different collection points. Calculate the *t*-value to determine if the null hypothesis is supported or not.

Results: Base of aquatic plants: 5, 8, 7, 3, 9, 5

Leafy foliage: 9, 8, 11, 7, 10, 8

P VALUE

The calculation for the *P* value assumes that the null hypothesis is true and estimates the probability that the measured result (difference between test group and control group) occurred due to the chosen sample. The calculation for *P* values includes the size in difference of results between two groups (effect size), sample size and the variations in the data. It uses these variables to evaluate random sampling errors. The statistical calculation produces a value between 0 and 1.00 and is interpreted as a percentage. For example, 0.025 is 2.5% probability; 0.42 is 42% probability. The *P* value is the probability that the result obtained (difference between the test group and control group) occurred due to random sampling error for a null hypothesis.

Examples of interpreting *P* values are as follows:

- A *P* value of 0.36 is interpreted as a 36% probability that sampling error caused the differences in results. That means that there is a 36% chance that the scientist's choice of samples included random sampling error and caused the measured differences between groups.
- A *P* value of 0.025 means there is a 2.5% probability that the different results between the groups was due to the chosen sample (sampling error). There is therefore a 97.5% chance that it is due to something else—natural variation, random error or even the independent variable.

Scientists use the *P* value to make conclusions about the reliability of their experiments.

The *P* value can only compare two sets of data. It does not compare an average (e.g. mean) or single values. It analyses the full set of measured results and compares it to another set. It is used to compare the control group (set of data) to a test group. It can also be used to compare a set of results for one test condition against another condition's set of results (e.g. the results from a change in the independent variable).

When the *P* value is determined, researchers specify the two sets of results used. There are numerous ways to communicate which results were compared using the *P* value, and the following examples illustrate a few.

It is not likely that *P* values will be used for student experiments, though they will be commonly encountered in research for the research investigation. In scientific research, the *P* value is often used to show significant results and then infer that the results are applicable to the general population. If a significant *P* value can be achieved, then a reliable methodology has been achieved, therefore it is assumed any sample in the population will achieve the same results. However, other statistical calculations must be present to conclude a significant result.

Significant *P* values and error

Commonly, *P* values of <0.05 are considered significant, though most biological research requires *P* values of <0.01 or even <0.001. This is because:

- it is just a calculation of probability and may not relate to the rest of the world's population or population of interest
- it only indicates sampling error and not the total of all errors.

The P value strongly indicates if further investigation is worthwhile. When accounting for all the other possible errors in scientific research and experimentation, the error rate (number of errors per experiment) has been noted as:

- when $P \leq 0.05$, 23–50% of experiments result in incorrect conclusions due to the accumulation of all the errors within the experiment
- when $P \leq 0.01$, 7–15% of experiments result in incorrect conclusions due to the accumulation of all errors within the experiment.

Interpreting *P* values

Figures 1.7.3 and 1.7.4 and Table 1.7.8 are results from research that has recorded significant P values. An interpretation has been provided for each figure to explain the meaning of the results.

Protein synthesis regulation example

As shown in Figure 1.7.3, leptin induces an increase in the synthesis of a certain protein (known as upregulation). The P value for the comparison between the control and leptin tests was significant, at ≤ 0.05. This means that there is a significant difference between the amount of protein synthesised between the control and the stimulation by leptin.

Understanding P values helps to interpret the results appropriately. The P value also assumes that any difference is due to sampling error. In this research, $P \leq 0.05$ infers that there is less than 5% probability that the difference in results is due to sampling error when the null hypothesis is true. In Figure 1.7.3, as there is a difference in the results when there shouldn't be (according to the P value null hypothesis), it is less than 5% likely due to sampling error. There is a greater than 95% probability that something other than sampling error caused the difference in results. In this investigation, the scientists are hoping it is the leptin.

Other possibilities need to be considered. Is it natural variation and just a typical and common result of nature? These results show error bars or 'whiskers', which are the standard deviation. The standard deviation shows that the natural variation between the two groups—control and leptin—do not overlap. As such, the two groups do not share results that are naturally the same.

The P value suggests that the difference between control and leptin is not a sampling error, and the standard deviation suggests that the difference is not natural variation. We can then surmise that the difference is either due to the variable, leptin, or other possible methodology errors.

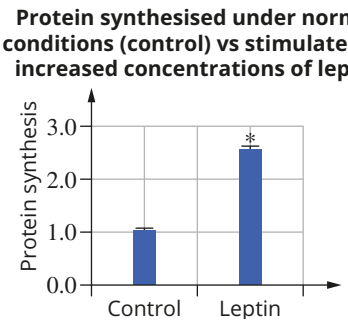


FIGURE 1.7.3 The amount of protein synthesised under normal conditions (control) and stimulated by increased concentrations of leptin. The asterisk (*) represents a P value less than 0.05 ($P < 0.05$).

Protein expression example

In this example, scientists investigated if heat shock protein (HSP 70) is expressed less by cells in the umbilical cord the further away the cells are from the insertion point. They measured how much was expressed by the cells 0–2 cm from the umbilical insertion point (where it attaches to the placenta), 2–4 cm from the insertion point and 4–6 cm from the insertion point. The scientists used the P value to compare a number of different sets of results. They compared the 0–2 cm results to the 2–4 cm results, the 0–2 cm results to the 4–6 cm results and the 2–4 cm results to the 4–6 cm results. To show which sets of data were compared, they used a line to refer to the sets of data.

In this investigation, only one comparison is considered significant: the P value of <0.01 assessing the difference between 0–2 cm and 4–6 cm (Figure 1.7.4). As P values assume the null hypothesis to be true, the P value estimates that there is a 9% probability that the difference in results between 0–2 cm and 2–4 cm was caused by random sampling error. This is not a significant difference. However, the results show that there is less than 1% probability that random sampling error caused the difference between the 0–2 cm results and 4–6 cm results.

Standard deviation (error bars) suggests that the difference in results between 0–2 cm and 4–6 cm were not caused by natural variation. Therefore, something other than natural variation caused the results.

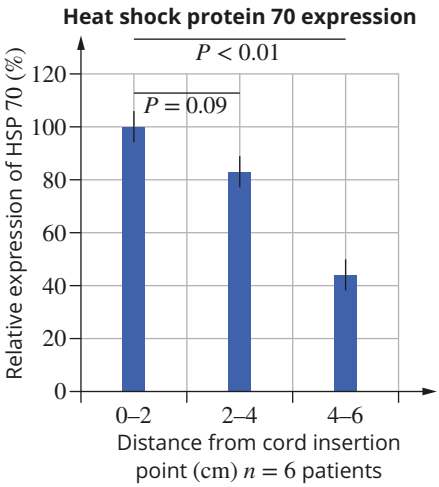


FIGURE 1.7.4 One method of displaying P values and the datasets that are being compared. When 0–2 cm and 4–6 cm were compared, a P value of <0.01 was calculated. Comparing 0–2 cm to 2–4 cm produced a P value of 0.09.

It could be inferred that as neither natural variation nor sampling error caused the results, they are the result of distance from the cord insertion point, or another variable not considered in this experiment.

An important factor must be considered. The sample size of six, shown in the label on the *x*-axis (n=6 patients), means six placental cords were used in this investigation. As standard deviation is heavily dependent on the sample size and the sample size is less than 20, the standard deviation is unreliable. This statistical calculation in this investigation is ineffective and does not provide effective evidence to infer or suggest a conclusion. Also, *P* values are dependent on sample size, along with other variables. The *P* value is therefore unreliable and it is ineffective at inferring or suggesting a conclusion. It is recommended that a larger sample size be tested to sample a larger proportion of the population and achieve more reliable values that are closer to the true value.

Sickle cell anaemia mutation example

In this example, scientists researched common variables in blood tissue components of patients with anaemia. They studied the components of blood such as red blood cell (RBC) count per litre, amount of haemoglobin, number of leukocytes, lymphocytes and platelets. Their aim was to see if patients with different variations of the BCL11A (rs4671393) sickle cell anaemia mutation would show differences in blood components. Their research was based on a null hypothesis that there would be no difference in the blood components between patients with different BCL11A (rs4671393) mutations.

Table 1.7.8 shows the results and where they used *P* values to determine if there was a significant difference. In this table, the researchers decided to show only significant *P* values. The significant *P* values were placed next to the variable that was significantly different to the others. The *CC* allele had a significant *P* value for RBC count (0.02), haemoglobin (0.02) and leucocyte count (0.01). The *CT* allele had a significant *P* value for lymphocytes (0.02) and platelet counts (0.02).

TABLE 1.7.8 Blood components of patients with BCL11A (rs4671393) mutations

Genotypes	Haematological variables									
	RBC (10^{12} L^{-1})		Haemoglobin (g dL^{-1})		Leucocytes (10^9 L^{-1})		Lymphocytes (10^9 L^{-1})		Platelets (10^9 L^{-1})	
Rs4671393 (BCL11A)	median	*P*	median	*P*	median	*P*	median	*P*	median	*P*
CC	2.9	0.02	8.1	0.02	13.1	0.01	6.1		316	
CT	2.7		7.8		12.6		5.1	0.02	393	0.02
TT	2.6		7.5		13.6		5.2		385.5	

This research studied the blood components from 610 patients, enabling an effective analysis and conclusive inference. Where there are significant *P* values, it can be concluded that the related blood component is significantly different and most likely not caused by random sampling error. It may be due to the independent variable, the BCL11A (rs4671393) mutation, or other variables such as instrumental error or another systematic error or an untested variable.

As no other statistical calculation was done (e.g. standard error or standard deviation) for many of these blood parameters, no other analysis can be done. It is also not known which two variables the *P* value is comparing. This means that a reason for the difference cannot be suggested, except that it is most likely not a result of random sampling error.

1.7 Review

SUMMARY

- The range is the difference between the highest and lowest values in a dataset.
- The mean, $\bar{x}$, is the average of a dataset. It is calculated from the formula:

$$\bar{x} = \frac{\Sigma x_1 + x_2 + x_3 + \ldots}{n}$$

  where

  Σ = sum of $x_1 + x_2 + x_3$ = measurement$_1$ + measurement$_2$ + measurement$_3$

  n = total number of measurements.
- The median is the middle value in an ordered list of values.
- The mode is the value that occurs most often in a dataset. The mode is particularly useful for describing qualitative or discrete data.
- Standard deviation is a measure of the spread or dispersion of data and its distance from the mean.
- The confidence interval estimates the reliability of an experimental method.
- Student *t*-tests assume that any variation between datasets is natural and not due to the independent variable.
- Student *t*-test values less than 0.05 are significant and are caused by the independent variable.
- *P* values estimate the probability that the difference between two sets of data is caused by random sampling error.
- *P* values are between 0 and 1.
- A *P* value less than 0.05 ($P < 0.05$) is considered significant.
- Significant *P* values mean the methodology process and sampling is reliable, and there is a good chance the independent variable is correlated to the difference in results.

KEY QUESTIONS

Retrieval

1 Describe a normal distribution.

Comprehension

2 Describe what the standard deviation informs scientists about a dataset.

3 Describe what the student *t*-test informs scientists about a dataset.

4 Describe what the standard error informs a scientist about a data test.

Analysis

5 Identify which of the following experimental results is most reliable.

A Mean 51 ± 4.2 g/d (CI 90%)
B Mean 51 ± 4.5 g/d (CI 90%)
C Mean 51 ± 5.2 g/d (CI 98%)
D Mean 51 ± 5.5 g/d (CI 98%)

6 For the dataset below, calculate and record the:

a range
b median
c mode
d mean and uncertainty

Data: 21, 28, 19, 19, 25, 24, 20

7 Infer what caused the difference in standard error.

	Quadrat A	Quadrat B
Number of samples	10	10
Mean (cm)	15.3	15.0
Standard deviation (cm)	2.9	5.1
Standard error (cm)	0.9	1.6

8 Infer what caused the difference in standard error.

	Quadrat A	Quadrat B
Number of samples	3	10
Mean (cm)	15.3	15.2
Standard deviation (cm)	2.9	3.0
Standard error (cm)	1.7	0.9

9 Draw a graph equivalent to Figure 1.7.2 that displays the standard deviation for class B from Table 1.7.4.

PART B STUDENT EXPERIMENT (IA2)

The QCAA stipulates that the student experiment be completed in Unit 3 Biology. The student experiment assessment task requires students to research a question or hypothesis. Students use research conventions to investigate the question or hypothesis by collecting, analysing and synthesising primary data. The experiment requires students to locate and use information beyond the scope of their knowledge and what they have been given.

The student experiment requires students to undertake the full scientific method. The QCAA Biology syllabus states that this process begins with a practical conducted during class, either a mandatory or a suggested practical. This in-class practical will be altered to conduct your own experiment. It is recommended that during the class practical you record your observations, queries and thoughts in a logbook. These notes can be used to lead to a research question for the student experiment.

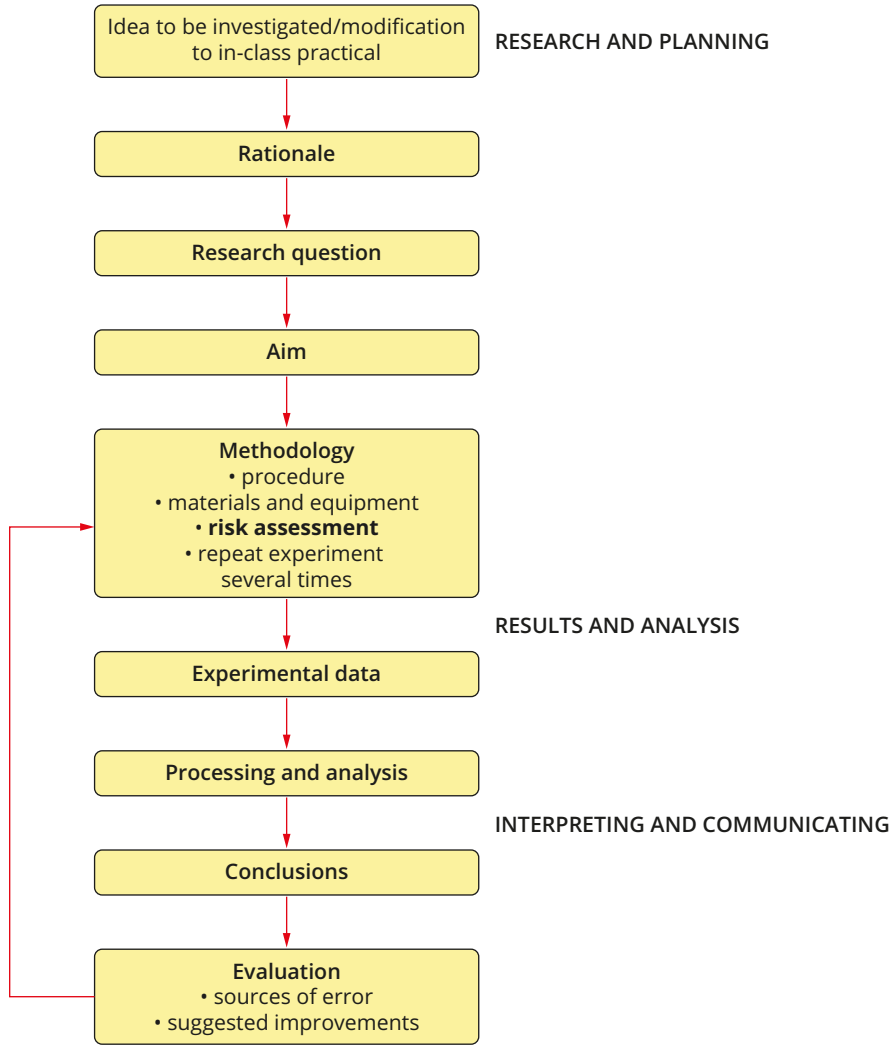
The student experiment constitutes 20% of the total assessment in biology.

A summary of the student experiment (ISMG IA2) is provided below. The summary includes the objectives and marking for this summative internal assessment. The student experiment may be presented in:

- written form (e.g. scientific report) of 1500–2000 words, or
- multimodal presentation form (e.g. poster presentation) of 9–11 minutes.

Criteria	Assessment objectives	Demonstrated by	Marks
research and planning	• apply understanding • investigate phenomena	• a considered rationale for the experiment • justifications for the experiment • a research question that is specific and relevant • collected data that is sufficient and relevant • considered risks and issues (ethical and environmental) and their management	6
analysis of evidence	• apply understanding • analyse evidence • investigate through experimentation	• use of relevant algorithms and correct data processing • detailed and careful coverage of relevant trends, patterns and relationships in the evidence • detailed and careful coverage of uncertainty and limitations of evidence • collection of relevant raw data and sufficient data	6
interpretation and evaluation	• interpret experiment evidence • evaluate experimental processes and conclusions	• a conclusion that is justified and addresses the research question • a discussion about the reliability and validity of the experiment that is supported by evidence • providing possible improvements and extensions to the experiment based on examination of evidence	6
communication	• present the experiment's findings, including methodology, conclusions, evaluation	• scientific language and representations that are concise and fluent • suitable use of genre conventions • appropriate referencing conventions to acknowledge sources	2
Total			20

The scientific inquiry is not a linear process. Scientists will not necessarily complete these steps in the stated order and some steps may need to be repeated or altered in order to more accurately address the research question, as demonstrated below.



Instrument-specific marking guide

Student responses are assessed against an instrument-specific marking guide (ISMG). In developing your student experiment and planning your response it is important to always have in mind the assessment objectives and, in particular, the characteristics that are described in the performance level descriptors.

The major features of the student experiment ISMG are outlined below and shown for the analysis of evidence criterion.

- The ISMG has four criteria: research and planning, analysis of evidence, interpretation and evaluation, and communication.
- Criteria are differentiated into performance levels, against which the qualities of the response are assessed.
- A performance level is comprised of a performance level mark, which may be a single mark or two-mark range, and performance level descriptor.
- The performance level descriptor describes the characteristics that are demonstrated by a response at this level.

The criterion 'Analysis of evidence' stipulates the characteristics of the top performance level. The interpretation of these characteristics is shown in the following table.

In order to be awarded a mark of 5 or 6 for this criterion, you need to show a thorough, thoughtful and comprehensive engagement with the task. For example, appropriately applying algorithms and representations of data through correct and relevant data processing is working at the highest performance level. However, adequate application and basic data processing is a demonstration of the lower performance level. If the application is rudimentary and data is incorrect and irrelevant, the performance level achieved is lower again.

Criterion: Analysis of evidence

Objectives

These are the objectives being assessed. Your work must demonstrate these objectives. Definitions of the objectives can be found in the syllabus glossary. How the objectives will be assessed is described below.

Descriptors

Each of the dot points in each performance level descriptor is called a descriptor. The descriptors contain all the characteristics required to achieve this level of performance. They outline the evidence teachers will search for in your work.

Performance level descriptor

The performance level descriptor is the whole of the left-hand cell in each performance level and is made up of all the descriptors in that level.

Objectives of assessment task

2 apply understanding of … to modify experiments and process primary data
3 analyse evidence from experiment
5 investigate … through an experiment

Key features that distinguish between marking levels	Marks
• processing data correctly and in a way that helps answer the research question • thoroughly identifying relevant relationships, trends, patterns to demonstrate systematic and effective analysis of evidence • thoroughly and appropriately identifying the uncertainty and limitation of evidence to demonstrate systematic and effective analysis of evidence • collecting sufficient and relevant data to demonstrate an effective and efficient investigation	5–6
• processing basic data to demonstrate an adequate application of algorithms and representations of data • identifying obvious relationships, trends, patterns to demonstrate effective analysis of evidence • rudimentary identification of uncertainty and limitations of evidence • collecting relevant data to demonstrate an effective investigation	3–4
• processing data incorrectly or processing data in a way that does not relate to the investigation • identifying incorrect or irrelevant relationships, trends, patterns to demonstrate ineffective analysis of evidence • identifying uncertainty or limitations of evidence incorrectly or to a degree that does not help resolve the investigation • collecting insufficient and irrelevant data to demonstrate an ineffective investigation	1–2
• descriptors not addressed	0

Performance level

Together, the performance level descriptor and the marks are known as the performance level. Once the most appropriate performance level has been identified (i.e. the one where most descriptors are matched) then your response can only receive one of the marks in the corresponding performance mark column. If all the descriptors of a performance level descriptor are demonstrated in your response, then the upper mark is awarded. Otherwise, the lower mark is awarded.

Performance mark

The mark awarded to the response for that criterion. It is related to the quality of the response as measured against the performance level descriptors.

In the modules that follow, you will find a guide to the scientific method (Modules 1.8 to 1.10) followed by an outline for producing a **scientific report** (Module 1.11).

Types of investigations

There are many different types of investigations that can be conducted in biology. You are probably most familiar with practical investigations or experiments. Other types of investigations involve researching and evaluating primary and/or secondary sources of information to answer a question. Examples of the types of different investigations are listed in the two tables below.

Examples of primary research with examples

Examples of primary research	Possible activities/tasks to be completed
conducting experiments in a laboratory	• planning a valid experiment • conducting a risk assessment • working safely • recording observations and results • analysing and evaluating data and information
conducting fieldwork	• conducting a risk assessment • working safely • recording observations and results • analysing and evaluating data and information
conducting surveys	• writing questions • collecting data • analysing data and information
designing a model	• identifying a problem to be modelled • summarise key findings • identify advantages and limitations of the model

Examples of secondary research with examples

Examples of secondary research	Possible activities/tasks to be completed
researching published data from primary and secondary sources	• finding published information in scientific magazines and journals, books, databases, media texts, labels of commercially available products • analysing and evaluating data and information

Before you are able to start the practical side of your investigation, you first need to understand the theory behind it. This section is a guide to some of the key steps that should be taken when you start to develop your research question.

Taking the time to carefully plan and design a practical investigation before you begin will help you maintain a clear and concise focus throughout. Preparation is essential. It is important to have both a solid understanding of the theory behind your investigation and a detailed plan for the practical components of your investigation.

In the following module, you will learn about the key steps you should take when planning and designing a practical investigation.

1.8 Research and planning

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- identify and explain the difference between controlled, measured, independent and dependent variables
- develop a research question or hypothesis
- evaluate research questions and hypotheses
- use a scientific journal to record experiments and experimental data
- plan, evaluate and refine scientific experiments
- explain what validity and reliability mean in relation to experimentation
- explain the difference between, and identify, qualitative and quantitative data
- characterise qualitative data as either nominal or ordinal
- characterise quantitative data as either discrete or continuous
- explain the difference between replication and repeat trials
- conduct risk assessments for planned experiments
- recognise common chemical HAZCHEM codes and symbols.

All scientific work begins with research and planning. This includes understanding the relationship between **controlled variables** and **measured variables** as well as the independent and dependent variable. Research and planning is the foundation of the scientific method and is always recorded in a journal. The journal will show a chronological record of ideas, development of knowledge and understanding, planning and refinement. The information recorded in the journal will be in chronological order rather than a logical conceptual order. The journal is an ongoing draft of scientific work from which the final scientific report is written.

IDENTIFYING AN EXPERIMENT AND DEVELOPING A RESEARCH QUESTION

Identifying an experiment for the student experiment requires you to **modify**, **refine**, **extend** or **redirect** a practical undertaken in class. Therefore, the experiment will be similar to the class practical but altered to investigate something slightly different, as shown in Figure 1.8.1.

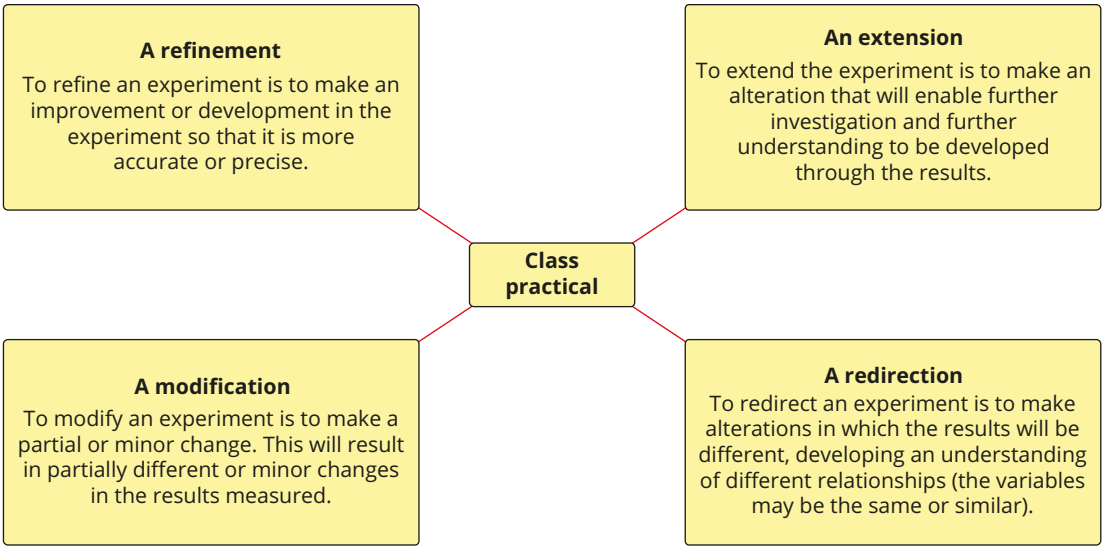


FIGURE 1.8.1 Chart showing possible changes to a class practical when developing a student experiment.

Identifying an experiment

During the class practical, record observations, points of interest, errors, ideas for improvement or variables that you believe are significant to the experiment. Choose one of these observations to alter. When choosing a topic or observation to alter from the class practical, consider the following:

- Choose an observation or topic that you already have some knowledge or understanding of.
- Choose an observation (variable) you find interesting.
- Check that your school laboratory has the resources for you to investigate the variable or perform the experiment.
- Choose a variable that can provide clear measurable data to demonstrate a relationship (e.g. the effect of variable y on variable x).
- Consider a null hypothesis.

Once you have chosen a variable to investigate, you will need to adapt the class practical by modifying, refining, extending or redirecting it to test your variable. Table 1.8.1 shows examples of modifying, refining, extending or redirecting practicals into a topic of interest for a student experiment.

TABLE 1.8.1 Some ideas for changes to class practicals

Class practical	Modification, refinement, extension or redirection	Student experiment idea
Determine the species diversity of flora in a 10 m × 10 m quadrat of bushland on the south-eastern border of the school.	modification and refinement	Determine the species diversity of flora along the 35 m south-eastern border of the school in a 2 m belt transect.
	modification and refinement	Determine the invertebrate species diversity of the 1.5 m *Grevillea* (Robyn Gordon) in the 10 m × 10 m quadrat on the south-eastern border of the school.
Collect and analyse primary biotic and abiotic field data using stratified sampling to classify the rocky shore ecosystem.	modification and refinement	Classify the zones in the rocky shore ecosystem using vertical stratification of biotic indicator species.
	extension	Investigate if a relationship exists between stratified zonation and salinity in rock pools.
	modification and redirection	Determine the species diversity in one stratified zone to evaluate the health of the ecosystem.
Analyse species diversity between the old cleared development site and the adjacent open eucalypt forest, appraising the 100 m belt transect with 5 m × 5 m quadrats at 10 m intervals.	extension	Repeat the class practical and modify the sampling technique to 10 random 5 m × 5 m quadrats for each site, comparing data.

The ISMG for the student experiment requires a justification for the modification to the methodology from the class practical. Recording all theory, conceptual understanding and reasons for the modifications in your journal as you learn and plan will provide the material to complete this descriptor.

The QCAA Biology syllabus requires the student experiment report to justify the alteration of the methodology from the class practical. When adapting the class practical into the student experiment, it is best to think of a single variable that may influence the outcome (independent variable). This may require some research. The more variables that are altered (including measured and controlled variables), the more complex the task becomes and the more research will be needed.

If only one variable is changed (the independent variable), the class practical can be used as the control and the data collected can be used to compare results. If both the independent and dependent variable are altered, the data between the class practical and student experiment is not comparable. The student experiment will need to determine its own control.

Defining the variables

The factors that can change during your experiment or investigation are called the variables. The experiment or investigation needs to determine the relationships between variables through the measurement of results. You should have only one independent variable, otherwise you cannot be sure which variable was responsible for the results. There are four categories of variables (Figure 1.8.2).

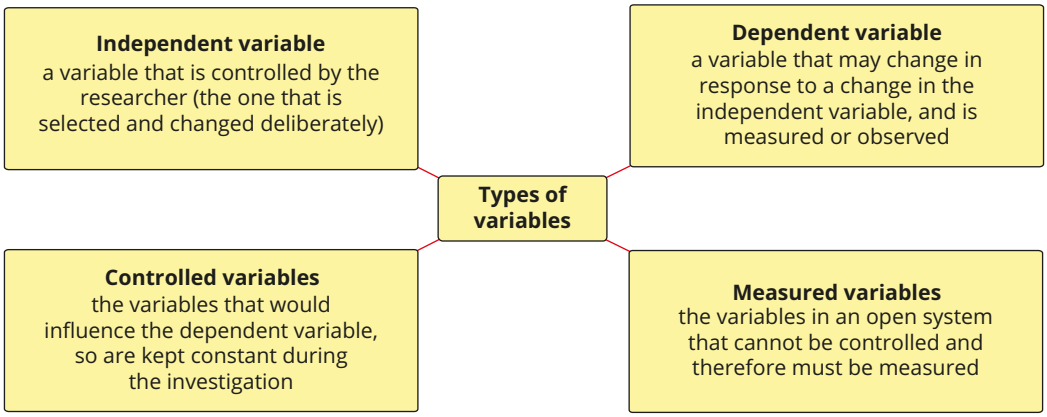


FIGURE 1.8.2 The four types of variables in experiments.

Developing a research question

A **research question** directs the scientific inquiry and the experiment. The purpose of your research question is to provide specific direction for the research, guide all inquiry and focus the analysis, interpretation and evaluation. It is the central foundation of your experiment or investigation.

The question determines the experiment and the experiment is testing the question. A research question should:

- be relevant to the class practical and have specific measurable variables defined
- clearly identify the subject matter of the experiment
- specify the scope or conditions of the investigation
- aim to find trends, patterns or relationships between two variables.

Consider the example in Figure 1.8.3.

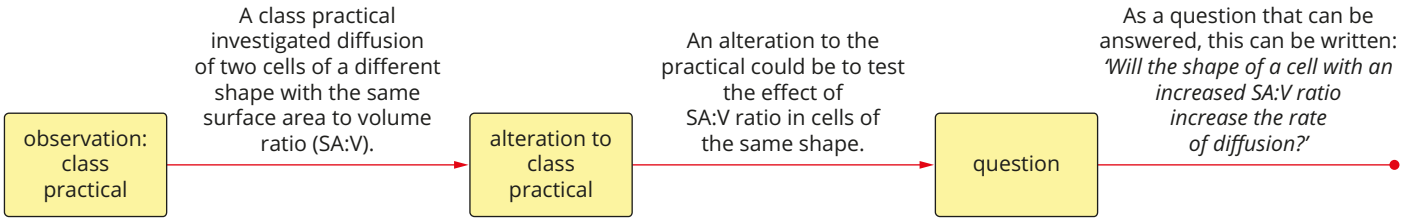


FIGURE 1.8.3 The process from class observation of a practical to altering the practical and developing a possible research question.

Background research for the student experiment would follow to refine the research question. The research could include:

- information about the variables
- correlations between variables
- ideas for refining the question.

Do not reject ideas that might seem improbable at this stage.

Figure 1.8.4 demonstrates the process of question refinement.

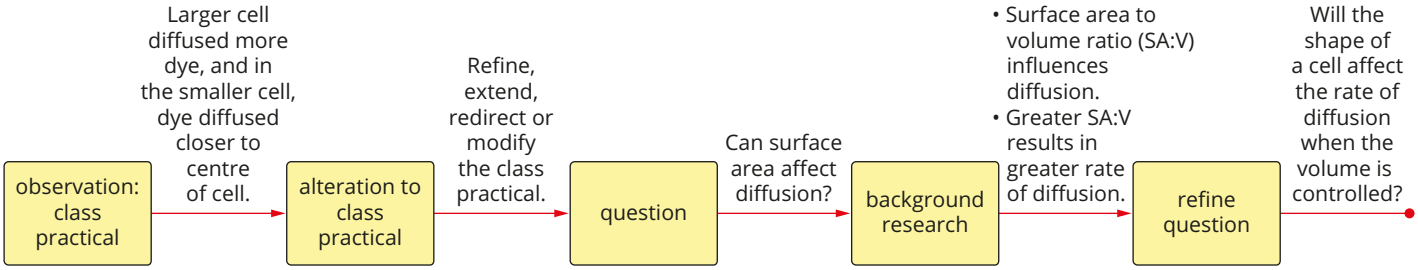


FIGURE 1.8.4 Refining the question.

The structure of a research question

All research questions should state the independent and the dependent variables. The question should ask if the independent variable will affect the dependent variable. The research question must specifically outline variables in a way that is measurable. This also allows the question to be answered.

Consider the following example:

	independent variable		dependent variable	
Will	species richness of detritivores at various heights	indicate	vertical stratification	?

The constructed research question should have the following characteristics:

- include measurable variables – the independent and dependent variable
- have a guiding word, such as *who, what, why* or *will*
- be phrased so that a definitive answer can be developed
- be able to link the guiding word to command verbs such as *identify, indicate, describe, compare, distinguish, analyse, evaluate* or *create* so that a task can be determined.

Table 1.8.2 gives examples of constructed research questions.

TABLE 1.8.2 Constructing a research question

Guiding word	Example research questions	What are you being asked to do? What are the command verbs?
What	*What* difference does surface area make to diffusion?	Identify and describe specific evidence, reasons and examples from a variety of possibilities. *Identify* and *describe*.
Will	*Will* natural antibiotics be more effective than synthetic antibiotics?	Identify and describe, giving reasons for effectiveness. *Identify* and *describe*.
How	*How* does the volume of cells of the same shape affect diffusion? *How* does substrate concentration affect enzyme activity? *How* do different wavelengths influence the number of chloroplasts in plant cells?	Identify and describe in detail a process or mechanism. Give examples using evidence and reasons. *Identify* and *describe*.
Why	*Why* do cells in different layers of a plant leaf have different concentrations of chloroplasts? *Why* is the relationship between enzyme activity and temperature non-linear?	Explain in detail the causes, reasons, mechanisms and evidence. *Identify* and *explain*.
Would	*Would* salinity affect prokaryotes and eukaryotes equivalently?	Evaluate. Justify, giving reasons for and against (using evidence and comparisons). *Evaluate* and *justify*.
Is/are	*Are* all prokaryotic cells smaller than eukaryotic cells? *Is* the cell wall of bacteria different from one species to another?	Evaluate. Justify, giving reasons and evidence. *Evaluate* and *justify*.
On what basis	*On what basis* is the substrate–enzyme complex a limiting factor in enzyme activity?	Evaluate. Justify, using reasons and evidence. *Evaluate* and *justify*.
Can	*Can* enzyme concentration increase the rate of enzyme activity?	Evaluate and assess. Is it possible? Give reasons, suggesting possible alternatives. *Evaluate*, *assess*, *justify* and *create*.
Do/does	*Does* surface area or volume affect the rate of diffusion?	Evaluate. Justify using reasons and evidence for and against. *Evaluate*, *assess* and *justify*.
Should	*Should* hands be washed with plain soap or antibacterial soap? *Should* hands be washed to reduce cross-contamination?	Evaluate advantages and disadvantages, implications and limitations. Make a judgement. *Evaluate*, *assess*, *justify* and *create*.

Formulating a hypothesis

From the research question, a **hypothesis** can be developed. A hypothesis is a statement that proposes a relationship between variables, because it is based on some level of understanding. This statement must be testable, meaning it must specifically and clearly state a change in variables that can be tested through measurement.

The student experiment does not necessarily require a hypothesis and they are not always appropriate or beneficial. In open systems such as ecological relationships, often a null hypothesis is the only option if a hypothesis is desired. A hypothesis, if it is suitable, requires the controlled variables to be more stringent during the experiment, resulting in an analysis of raw data that can specifically address the original inquiry of the observation. In an open system, it requires the observation and measurement of a vast number of variables to allow appropriate analysis, which usually involves statistical calculations. With more stringent controls, it may be possible to reduce errors and uncertainties, and the interpretation of results may be more straightforward; however, this is not always the case.

Because the hypothesis proposes a specific relationship between the independent and dependent variables, the hypothesis will either be supported or refuted by the results. To propose a specific relationship, the scientist must have some knowledge and understanding of the variables before writing the hypothesis.

To develop a hypothesis, similar steps are undertaken to developing a question. Steps for formulating a hypothesis are shown in Figure 1.8.5.

> The writing of a hypothesis is an optional inclusion that might support the development of your research question. A hypothesis is not an assessed component of the ISMG, and not every research question will lead to the development of a hypothesis.

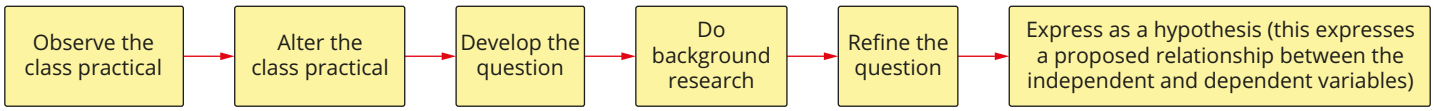


FIGURE 1.8.5 Process for formulating a hypothesis.

To formulate a hypothesis, write it in terms of the dependent and independent variables:

independent variable — dependent variable

If *x* happens, then *y* will happen.

Consider the following example:

independent variable — dependent variable

If species richness of detritivores is determined at incremental heights, then vertical stratification will be observed.

The 'if' part of the hypothesis refers to the independent variable, which is the variable you alter in the experiment or specifically observe at various increments. The 'then' part relates to the dependent variable, which is the variable you hope to observe change in, relative to the independent variable.

A hypothesis does not need to include 'if' and 'then' in its wording. For example, the previous hypothesis could also be worded in the following ways:

- Example 1 – Vertical stratification will be observed when the species richness of detritivores is determined at incremental heights.
- Example 2 – When species richness of detritivores is determined at various heights, corresponding vertical stratification will occur.

It is often easier to evaluate a null hypothesis through investigation and data analysis in an open system. Here is an example:

independent variable — dependent variable

Vertical stratification will not be observed when species richness of detritivores is determined at incremental heights.

A good hypothesis can be tested to be true (verified or supported) or false (falsified or refuted) by investigation.

EVALUATING YOUR RESEARCH QUESTION

The quality of the research question is vitally important in determining the quality of the investigation and scientific report.

Use the prompts in Figure 1.8.6 to evaluate your research question and guide your refinements.

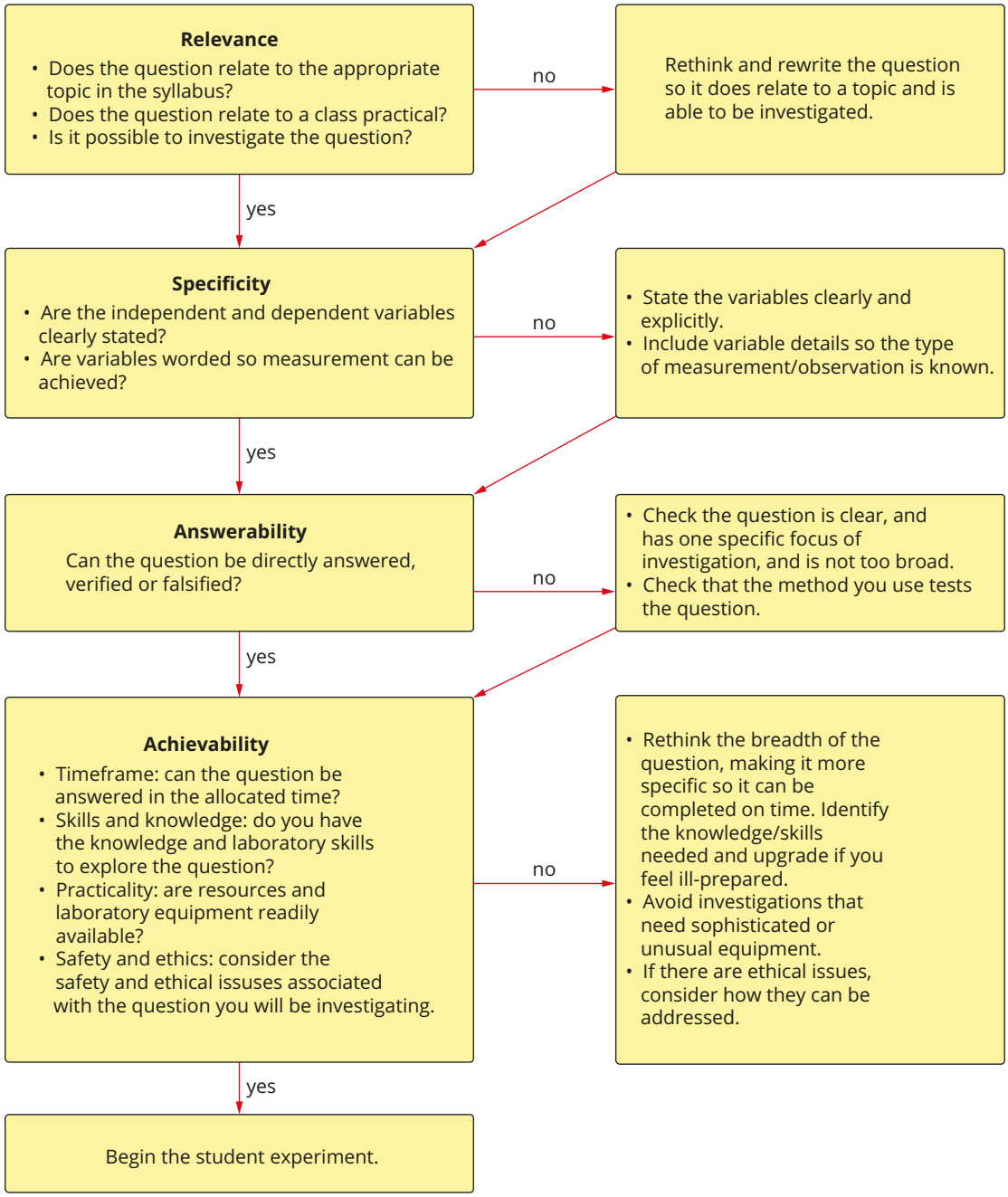


FIGURE 1.8.6 Summary of steps for evaluating a research question.

Examples of research questions and hypotheses

Table 1.8.3 demonstrates that the student experiment may be expressed as either a question to be investigated or a hypothesis.

TABLE 1.8.3 Student experiments expressed as research questions and hypotheses

Research question	Hypothesis
Does flora biodiversity decrease as the distance from a river bank increases?	As the distance from the river bank increases, the biodiversity of flora will decrease linearly.
Will a transect line survey determine species diversity equivalently to random quadrat sampling for undisturbed and disturbed dry sclerophyll forest?	A transect line survey will not determine species diversity equivalently to random quadrat sampling of undisturbed and disturbed dry sclerophyll forest.
Will species diversity differ between the detritus material in the school garden at the north-east corner of the sports oval and the undisturbed bushland to the east of the school?	There will be no difference in species diversity between the detritus material of the school garden at the north-east corner of the sports oval and the undisturbed bushland to the east of the school.

DEVELOPING THE RATIONALE

Once you have decided on a research question, you will need to develop a rationale explaining the conceptual relationship between the variables in the research question. The rationale will also include the scientific reason for the modification to the class practical. It is developed by completing relevant background research. When the scientific report is written for the student experiment, your rationale will be included.

Researching relevant scientific information

The student experiment in the QCAA Biology syllabus requires you to:

- research what is currently known about the relationship between the dependent and independent variable
- develop a methodology that allows sufficient, relevant data to be collected that enables the research question to be answered
- manage the risks and issues associated with the experiment.

In your background research for the student experiment (IA2), you are required to demonstrate:

- a rationale for the experiment that demonstrates consideration
- methodological modifications that have been justified
- methodology which collects data that is pertinent to, and adequate to test, the research question, with deliberate design.

Furthermore, the QCAA Biology syllabus expects background scientific information to be used in a rationale for the experiment to:

- explicitly justify the modifications to the methodology (alterations to the class practical)
- explain how the methodology will enable the research question to be answered through the collection of the data
- inform risk, ethical and environmental management. This relates to identifying how the risks associated with the experiment will be mitigated through personal protective equipment or specific features of the methodology. The ethical and environmental issues could relate to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes or the Animal Ethics Committee for each use of 'animals' or type of use of 'animals'.

The rationale is also expected to inform the interpretation of the evidence (results) and conclusion.

The interpretation of evidence (the results) and conclusion applies the same conceptual understanding used in the rationale. It is not expected that the data will necessarily concur with the conceptual explanations, rather that the analysis, interpretation and conclusions will address or acknowledge the concepts and explanations introduced in the rationale.

All the expectations and characteristics outlined in the syllabus are dependent on or extended from the research question. Ensure all background research is directly related to the research question and the relationship it details. That is, the relationship between the independent and dependent variables and how they relate. That way, knowledge and understanding required to complete the student experiment will be developed. This will keep the research relevant, which is essential. Also, note that the rationale for the experiment will be limited by the word count, accentuating the need to provide only relevant background information.

There is so much information to record during a student experiment that it would be best to keep it all in a single document. Scientists record all their ideas, questions, background research and literature reviews, methodology drafts and revisions, results, refinements etc. in a single document called the **scientific journal**.

The scientific journal is comprehensive and includes all formal and informal information. During the scientific method, especially in the early stages of developing a research question and planning a methodology, it is not known how vital some information will become throughout the process. The information in the journal will be used in a rationale for the experiment to explicitly justify the modifications to the methodology (alterations to the class practical), provide a reason for collecting data and inform risk, ethical and environmental management. It is also expected to inform the interpretation of the evidence (results) and conclusion.

A scientific journal should not be included in the assignment submission and should not be used to earn marks. If it is, it will be included in the word count. Due to the complexity and size of the student experiment assignment, use the scientific journal to record all information, planning, drafting etc. to enable you to produce a final product—the scientific report—which has been processed and fine-tuned.

PLANNING AND REFINING METHODOLOGY

This section is a guide to some of the key steps that should be taken when planning and refining an investigation.

Planning experiments

Once you have formulated your research question, developed an understanding of concepts and relationships between the variables, you will need to plan your experiment. You will also need to consider the ethical and safety implications of the testing during the experiment.

The methodology of your experiment is a specific step-by-step procedure. However, it may be written in paragraph form in the final scientific report. You must ensure that the methodology is valid, specific, reliable and accurate. All of these factors need to be considered when planning.

Create a work schedule that outlines the time frame of your experiment (including all trials and/or samples), being sure to include sufficient time to repeat experiments if necessary. Ten hours of class time will be made available to you and you can also use your own personal time. Check with your teacher that your methodology is appropriate, and that others will be able to repeat your experiment exactly by following the methodology you have written. If you have planned well, you will be able to test your methodology and run trials. You need to be able to perform your experiment independently, in the time available in the school laboratory, with minimal support from your teachers and school laboratory staff.

Validity

Validity refers to whether an experiment or investigation is in fact testing the set research question or hypothesis. Is the experiment obtaining data that is relevant to the question?

Factors influencing validity include:

- whether your experiment measures what it claims to measure, as determined by the research question or hypothesis

- the certainty that something observed in your experiment was the result of your experimental conditions and not some other cause that you did not consider (in other words, that the measurements and tests are what they were designed to be)
- (if possible, but not necessary for the student experiment) the degree or scope to which your findings can be generalised to the wider population from the sample measured. This is dependent on sample size and sample selection, which may not be possible.

To ensure an investigation is valid, it should be designed so that only one variable is being changed at a time. The remaining variables must remain constant so that meaningful conclusions can be drawn about the relationship between variables.

Also, the raw data collected during the experiment must be appropriate to ensure the data is valid. To ensure validity, carefully determine:

- the controlled and/or measured variables
- the appropriate raw data to be collected (quantitative versus qualitative), and that each will be measured, collected or controlled appropriately.

Data can be either qualitative or quantitative. **Qualitative data** is descriptive and unmeasurable and uses descriptions or adjectives to record observations. **Quantitative data** is empirically measurable and uses instruments to record observations as numbers with units.

Qualitative data can be further described as nominal or ordinal, and quantitative data can be further described as discrete or continuous (Figure 1.8.7).

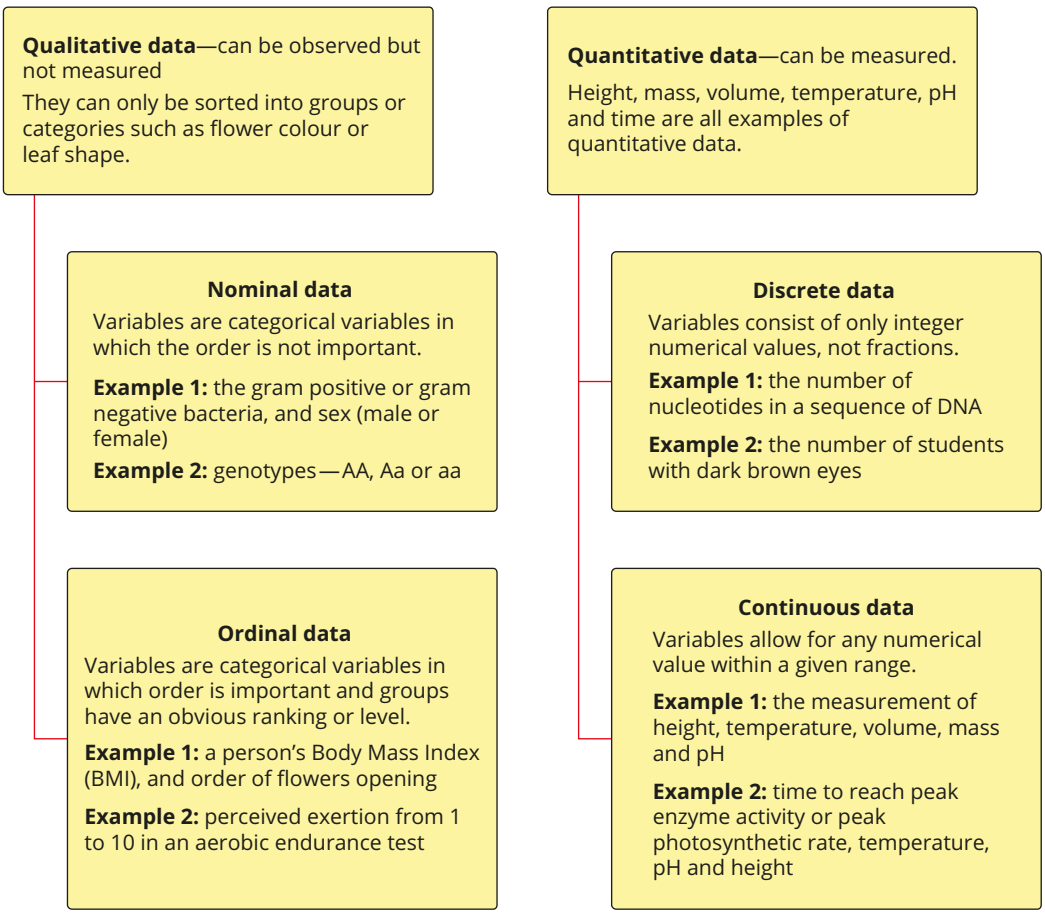


FIGURE 1.8.7 Qualitative and quantitative variables.

When planning the methodology, it is not appropriate to record temperature as qualitative data (e.g. cold, warm or hot). Temperature should be measured using an instrument and recorded as quantitative data (Figure 1.8.8 on page e66).

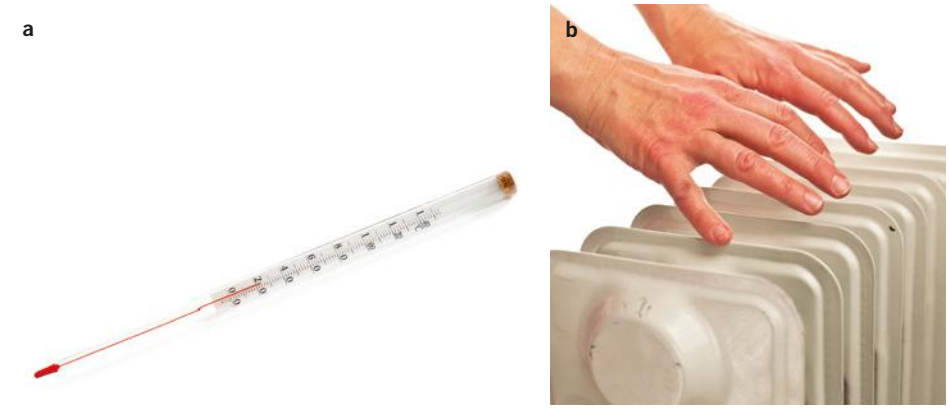


FIGURE 1.8.8 (a) A thermometer or a wireless temperature sensor will measure temperature empirically and provides quantitative data that can be analysed statistically. Processing empirical data can produce discrete, explicit and comparative analysis. (b) Feeling heat radiating from a heater is an example of qualitative data, as it is based on personal observation. Qualitative data cannot be statistically analysed.

When planning, consider the most appropriate way to record data. Depending on the experiment, it may be through qualitative observation (e.g. colour of leaves) or measuring the quantitative results of wavelength (e.g. light reflected from an object) (Figures 1.8.9 and 1.8.10).

FIGURE 1.8.9 (a) When recording the colour of leaves using qualitative data, a reference image like this one helps to record good qualitative data. (b) Colour can also be measured using a spectrophotometer, which measures the wavelength emitted or reflected by the object and produces quantitative data.

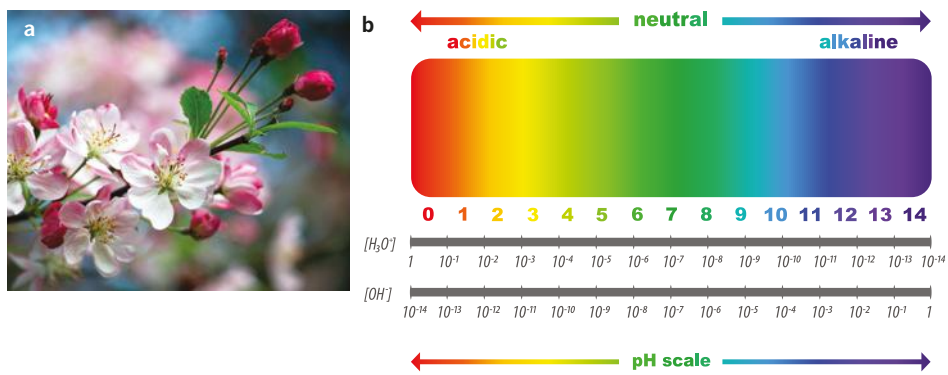


FIGURE 1.8.10 (a) Recording the order in which flowers open is an ordinal variable that is qualitative data. (b) The pH scale can be measured numerically (empirically) on a continuous scale from 1 to 14. This is a continuous variable that is quantitative data.

Controls

Controlling as many variables as possible to determine what has influenced the results provides more accurate and precise data. In open-system investigations such as ecological experiments, it is impossible to control variables. Measuring and recording observational data under natural influences and variables also influences the validity and reliability of the data.

Some natural variables that influence measurements and data are time of day, temperature, amount of light, season and level of noise. A methodology to overcome this is to observe and investigate two groups: a **control group** and an **experimental group**. The control group observes and measures the population (or sample) under natural conditions. The experimental group is identical in every way to the control group except for the single experimental (independent) variable that is observed or measured. This is a controlled experiment. This allows the examination of the independent variable, which is required to validly answer a research question or test a hypothesis.

Randomisation

Random selection of your sample reduces selection bias, reduces uncertainty (example standard error) and improves validity. Selection bias occurs when the sample doesn't reflect the wider population. For example, if you were scoring phenotypes in large trials of genetically modified plant crops, it is more valid to choose locations at random throughout the plot than one in which you choose only the edges of the plot. Random selection encourages the collection of data or evidence from samples that will more likely include the true value (Module 1.5). Random selection of samples should result in samples that reflect natural variation.

If the experiment is a closed system and controlled, the controlled variables need to reflect natural conditions as much as possible to minimise selection bias of conditions, increasing systematic error and influencing the relationship between the tested variables.

Reliability

Reliability is the ability to obtain equivalent results when an experiment is repeated (Figure 1.8.11). The closer the results are to the true value, the more reliable it will be. This is because it will be more likely to achieve equivalent results when the experiment is repeated. In an open-system investigation, each measurement or observation could be affected by errors. It is therefore beneficial to plan for **replication** of samples and **repeat trials** to improve reliability.

It is especially important for an open-system investigation to strive for reliability. Biological investigations that involve numerous individuals will always be influenced by natural variation. Rather than controlling natural variation (which is not possible), it is imperative to accept and incorporate it into the planning, measurement and interpretation of data. Biological experiments or investigations need to account for natural variation to try to measure the true value for the population mean. The more the investigation results reflect nature, the more reliable the experiment will be.

To improve reliability, you should:

- choose an appropriate sample of the population (including sample size and selection of individuals)
- specify the materials and methods in detail (including precision and uncertainties in measurement)
- include replicate (several) samples within each experiment or several observations within an investigation
- take repeat readings of each sample
- run the experiment or trial more than once.

Sample size is extremely important in scientific experiments. The sample size affects the:

- representation of the phenomenon
- natural variation, errors and uncertainty
- results.

Reliability is improved by replication and repeat trials. Replication means using multiple samples in an experiment. Repeat trials means repeating the experimental test.

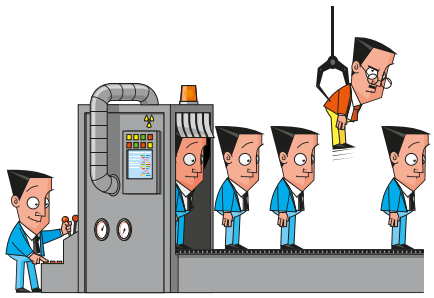


FIGURE 1.8.11 If you can reproduce your results, they are reliable.

A larger sample size offers more evidence to support the experimental results and helps repeatability and therefore reliability. The greater the sample size, the more reliable the data. Also remember that accuracy and precision are important in obtaining reliable data (Module 1.5).

Sourcing appropriate equipment and materials

You will need to decide on the materials, technology and instrumentation that will be used to carry out your student experiment (investigation) to achieve reliable results. It is important to find the right balance between items that are easily accessible and those that will obtain accurate and precise results. When conducting your investigation, the precision of the chosen instrumentation and how this affects the accuracy and validity of the results will have to be recorded in the journal for the scientific report.

ELECTRONIC DATA ACQUISITION IN SCHOOLS

Data collection in recent times has significantly improved with electronic data acquisition. The ability to connect independent sensors to any device has enabled simple and highly effective electronic data acquisition over extensive periods of time.

Data acquisition can be achieved using sensors or probes, recording data to a standalone device, computer, tablet (Figure 1.8.12) or even a phone. Many smart watches and apps in phones (Figure 1.8.13) include digital sensors that can be accessed and used for some class practicals and experiments. The software will often graph the data and perform the uncertainty propagation automatically. This specialised software allows for many analysis functions not found in standard spreadsheets.

FIGURE 1.8.12 Data acquisition software produces real-time graphs that can be downloaded or printed.

FIGURE 1.8.13 Many smart watches and phones have digital sensors and inbuilt data loggers.

Electronic data acquisition and data logging

Electronic data acquisition is highly precise and accurate. There are many sensors, probes and instruments available that can measure a vast variety of phenomena in a single device. Some examples of data acquisition devices are shown in Figure 1.8.14.

Often one of the measurements is time. The user decides what the other measurement (or variable) is by selecting the type of sensor or probe to use. Probes can measure temperature, distance, water content, electrical quantities, concentration of gases in air, loudness of sound, the amount of pollutants in the environment and many more phenomena.

FIGURE 1.8.14 (a) Sensors connected to computing devices such as phones and tablets are readily used in the field. (b) Many different probes and sensors can attach to a single device, such as those being used for water quality measurements here. (c) They can be very precise and measure multiple times per second, such as the frequency and amplitude of air vibrations in sound. (d) They are usually easy to use and produce results immediately, for example, providing the temperature of skin upon contact.

Recording can be continuous or manual, and the measurements are saved electronically. The saved measurements can then be accessed via a computer or directly from the tablet screen.

The rate at which measurements are recorded is called the sample rate (data capture rate). Data capture rate can be varied from as short a time as 1/100 000 of a second through to once an hour. The data capture rate selected will depend on what the operator needs.

RISK ASSESSMENT

While planning for an experiment or investigation in the laboratory or outside in the field, it is important for your safety and the safety of others that you consider the potential risks.

Everything we do involves risk. **Risk assessments** identify, assess and control hazards (risks with potential to harm). A risk assessment should be performed for any situation, whether in the laboratory or in the field, that could harm people or animals. Always identify the risks and control them to keep everyone safe (Figure 1.8.15).

FIGURE 1.8.15 When planning an investigation, you need to identify, assess and control hazards.

To identify risks, think about the following:

- the activity that you will be carrying out and all the individual tasks
- where in the environment you will be working, for example, in a laboratory, school grounds or a natural environment
- how you will use any equipment, chemicals, organisms or parts of organisms that you will be handling
- what clothing you should wear, for example, lab coat and goggles.

The hierarchy of risk controls is shown in Figure 1.8.16. It is organised from the most effective to least effective. The most commonly used risk control measure that addresses most risks is personal protective equipment (PPE). The least common, but most protective, control measure is eliminating the risk from the scientific investigation.

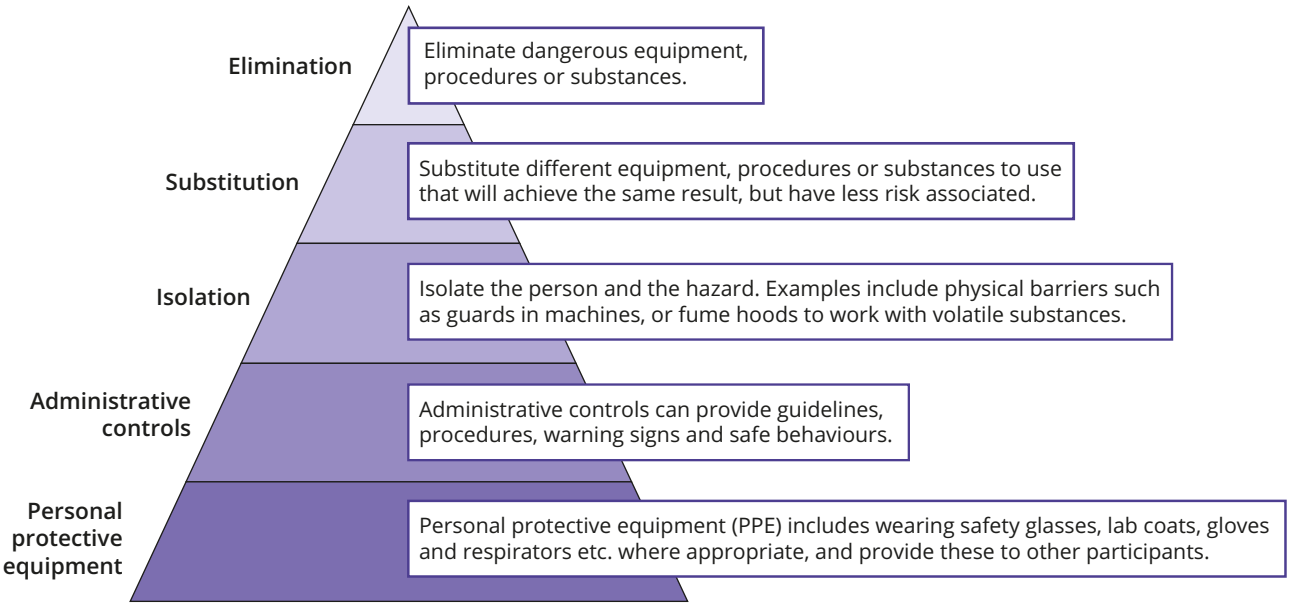


FIGURE 1.8.16 The hierarchy for risk control is shown in this pyramid, progressing from bottom to top in order of increasing importance.

Personal protective equipment

Everyone who works in a laboratory wears items that help keep them safe. This is called **personal protective equipment** and includes:

- safety glasses
- shoes with covered tops
- disposable gloves for handling certain chemicals
- a disposable apron or a lab coat if there is risk of damage to clothing.

Some PPE is shown in Figure 1.8.17.

FIGURE 1.8.17 Personal protective equipment includes protective eye wear, lab coats and gloves.

Chemical codes

The chemicals at school or the hardware shop have a warning symbol on the label. These are **chemical (HAZCHEM) codes**. In 2017, the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) was introduced in Australia for use in workplaces, including school laboratories. Some common warning symbols and their meanings are shown in Table 1.8.4.

TABLE 1.8.4 Common warning symbols and their meanings

Symbol	Meaning
	Corrosive: Can dissolve or eat away at substances, including tissues such as your skin or airways
	Acute toxicity: Can cause injury or death if ingested, inhaled or absorbed
	Health hazard: Causes discomfort, pain or itchiness
	Flammable: Is flammable
	Chronic health hazard: Includes biological harm, for example, cancer, allergy, breathing difficulties

Safety data sheets

Each chemical substance has an accompanying document called a **safety data sheet (SDS)** (Figure 1.8.18). An SDS contains important safety and first-aid information about each chemical you commonly use in the laboratory. If the products of a reaction are toxic to the environment, you must pour your waste into a special container (not down the sink).

The SDS provides employers, workers and health and safety representatives with the necessary information to safely manage the risk of hazardous substance exposure.

RISK ASSESSMENT [redacted]

Bacteria in yoghurt

Written by: Mr Smith **Commenced on:** 25 Jul 2017 **Expires:** 25 Oct 2018

Classes for which experiment is required
Teacher: Mr Smith **Year Group:** 11 Biology

Room	Period	Date
L1	1	Mon 1/1/18

Items to be prepared by laboratory technician

Wire inoculating loops 10
Milk nutrient agar plates 20
Plain home brand yoghurt 10x 3mL in test tubes/microtubules with lid
Tape for sealing agar plates
Permanent markers (black) 10
Box of matches 10 OR spark lighters 10
Incubator set at 25 degrees
Spray bottles of 70% ethanol 5

Procedure or reference, including variations
Senior Biology

Equipment to be used

adhesive tape
Potential hazards
Do not tape over mouth or nose, or use as a restraint.

box of matches
Potential hazards
Box burns violently if ignited.
Standard handling procedures
Keep dry. Used matches should never be returned to the box. Count boxes out and in.

bunsen burner
Potential hazards
Roaring flame is very hot and can cause severe burns. Rapid passage of hand through fully luminous flame usually does not result in a burn. Roaring bunsen burner may "burn back" at low gas flow, with flame emerging from air holes in base; this makes the base of the burner hot to touch and liable to cause burns. Gas from gas tap or from end of rubber tube burns with large luminous flame, likely to cause burns. Rubber hose is easily melted by flame from burner, e.g. if burner knocked over, resulting in fire from burn hole in tube. Ensure hair is tied back, so does not catch alight.
Standard handling procedures
Inspect and clean the jet and base of bunsen burners regularly. Inspect and replace tube whenever any sign of wear or damage is noticed. Use only hoses of the correct size to ensure a comfortable fit on both bunsen burner and gas tap.

hand sanitiser

incubator
Potential hazards
Possible source of electrical shock if not wired correctly. Possible ignition source.
Standard handling procedures
Check for electrical safety each time before use. Test and tag at regular intervals.

inocculation loop
Potential hazards
Loop will be hot following flame sterilization and may cause burns. When flaming the loop, ensure the loop is held at a downwards angle to reduce the amount of microbial aerosols created.
Standard handling procedures
Ensure no flammable liquids near flame during sterilization. Ensure loop is flame sterilized before and after use. Do not inoculate unknown organisms since they may be pathogenic.

marker pen
Potential hazards
Inhaling the contents may be harmful due to the volatile solvents. Flammable.
Standard handling procedures
Recap tightly after use. Do not allow students to inhale fumes.

test tube, small (~75 x 8 mm), soda glass
Potential hazards
Breakage of test tubes. Cuts from chipped test-tube rims. Melts at red heat in roaring bunsen flame (sodium fusion test) and shatters when dropped into water; use gauze to protect against flying glass fragments.
Standard handling procedures
Inspect and discard any damaged test tubes. Sweep up broken glass with brush and dustpan; do not use fingers.

Chemicals to be used and produced

agar, solid
Class: nc PG: none **(K-12)** Users: (1,2,3,4,5,6) CAS: 9002-18-0
GHS data: Not classified as a hazardous chemical.
Potential hazards
Low toxicity.

ethanol 5-13 M (24-70% wt/wt) (ethyl alcohol) $CH_3CH_2OH_{(aq)}$
Class: 3 PG: III **7-12** Users: 1,2,3,4,5 UN: 1170 CAS: 64-17-5
GHS data:
DANGER Flammable liquid and vapour
Causes eye irritation
Potential hazards
FLAMMABLE. Liquid irritates eyes. Prolonged contact with skin causes irritation. Low toxicity. Higher concentrations form violently explosive mixtures with nitric acid and other oxidising agents. Reaction of 70% wt/wt ethanol with acidified dichromate solution is highly exothermic. Potassium reacts explosively with aqueous ethanol.
Standard handling procedures
70% wt/wt ethanol is the optimum concentration for killing bacteria and is often used as a sterilizing liquid. Ethanol is a controlled substance, not usually available in schools. Aqueous methylated spirits is the usual substitute in schools.

Living organisms to be used

bacteria
Potential hazards
Possibility of infection during experiments with unknown bacteria. Some bacteria are highly pathogenic.
Standard handling procedures
Some school authorities ban the culturing of unknown micro-organisms in experiments, due to the possibility of culturing pathogens. Many school authorities do not permit sub-culturing of bacteria from wild cultures. Your school authority may allow commercially obtained pure strains of non pathogenic bacteria to be subcultured from one plate to another, provided the appropriate safeguards are followed. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

yoghurt
Potential hazards
ALLERGY ALERT. Some individuals are allergic to dairy products. Do not eat in Science laboratory, due to the possibility of chemical contamination.
Standard handling procedures
Store in refrigerator for maximum of 1 week or until expiry date.

Others
Students will wear safety glasses throughout the procedure.
spark lighters - overuse will damage flint; caution to be issued.
milk allergies - will not be ingested; caution students with allergies.

Knowledge
I have read and understood the potential hazards and standard handling procedures of all the equipment, chemicals and living organisms.
I have read and understood the (Material) Safety Data Sheets for all chemicals used and produced.
I have copies of the (Material) Safety Data Sheets of all the chemicals available in or near the laboratory.

FIGURE 1.8.18 Two pages from a risk assessment for a bacterial experiment that uses the safety data sheet to alert the reader to any potential hazards, including appropriate measures to reduce risk of harm.

Identification keys

Identification keys are used primarily for identifying species and organisms in an ecological investigation. They are also useful for identifying organisms that can cause harm or may pose a risk. Any ecological survey should use an identification key. For the student experiment, using one that also identifies risks, or adding your own notes to the key to identify risks, should be considered as part of the risk assessment.

ETHICAL CONSIDERATIONS

When planning an investigation, you should identify all possible ethical considerations and evaluate their necessity or ways to reduce or mitigate them. **Ethics** is a set of moral principles by which your actions can be judged as right or wrong. Every society or group of people has its own principles or rules of conduct.

Scientists have to obtain approval from an ethics committee and follow ethical guidelines when conducting research that involves animals, including, and especially, humans. Ethical issues could include the following:

- How can this affect the wider society?
- Does one party benefit over another, for example, one individual, a group of individuals or a community?
- Is there a risk of physical or mental harm to people involved in the research?
- Does the research prevent anyone from gaining their basic needs?
- How can this impact on future ethical decisions or issues?
- Does the research cause damage to the environment?
- Does the research cause harm to other living things?

In reality, school biology investigations will generally have minor ethical issues, if any, but you should consider these in your planning.

Scientific research involving humans or animals must be approved by an ethics committee before it can commence. All research involving animals in Australia must comply with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. However, there might still be public concern about some types of research. The use of live animals in research (for example, for testing the safety of pharmaceutical products) is also an issue for many people.

REFINING THE METHODOLOGY

As the planning of the methodology is not linear, refinement may occur several times (due to further background research, refining the research question or as variables become increasingly understood). Scientists take several years to refine their methodology.

Record all refinements in the journal. The following list may help with refining the methodology.

- Record everything.
- Be prepared to make changes and refinements to the plan and methodology.
- Note any difficulties encountered and the ways they were overcome. What were the failures and successes?

Every test carried out can contribute to the understanding of the investigation as a whole, no matter how much of a disaster it may first appear. Figure 1.8.19 shows a student journal demonstrating basic developments and refinements in their methodology.

If the expected data is not obtained, don't worry. As long as it can be critically and objectively evaluated, the limitations of the investigation can be identified and further investigations proposed, the work is worthwhile. Sometimes a different point of view, such as from a fellow student or a teacher, is all that is needed to find a solution. An evaluation that includes suggested improvement to the methodology or experiment is required in the scientific report. In the scientific report, you will have to justify your modification.

> It is common for experimentation and testing not to occur according to plan. It is vital that comprehensive background research has been undertaken. Refinements are often made during experimentation to improve validity and reliability. This may be due to time constraints, instrumental limitations or resource limitation. Refinements also reduce error and uncertainty as the experimenter becomes aware of these problems.

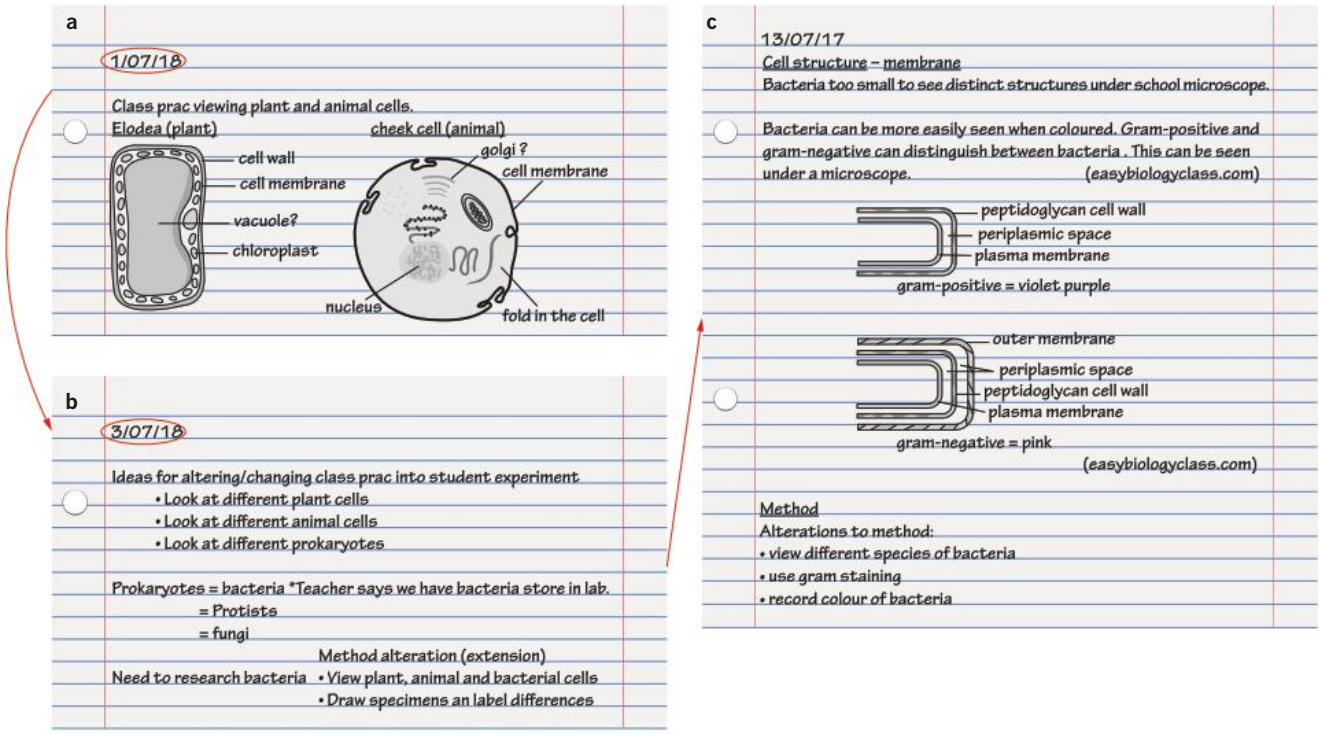


FIGURE 1.8.19 Demonstrating the alteration and refinement of a class practical into a student experiment in a student's journal. (a) The observations from the class practical. (b) The recordings of ideas and possible alterations to the class practical, developing a student experiment. (c) The refinement of the student's idea for the experiment. The refinement was based on research.

1.8 Review

SUMMARY

- An independent variable is a variable that is controlled by the researcher.
- A dependent variable is a variable that may change in response to a change in the independent variable, and is measured or observed.
- Controlled variables are kept constant during the investigation.
- Measured variables cannot be controlled and must be measured.
- Research questions should have the following characteristics:
  - measurable variables (the independent and dependent variable)
  - a guiding word, such as *who*, *what*, *why* or *will*
  - be phrased so that a definitive answer can be developed
  - be able to link the guiding word to command verbs so that a task can be determined.
- A simple way to formulate a hypothesis is to link the independent and dependent variables using the following sentence structure: If (independent variable) happens, then (dependent variable) will happen.
- A scientific journal is a document scientists use to record their ideas, questions, background research and literature reviews, methodology drafts and revisions, results and refinements related to an experiment.
- Validity refers to whether an experiment or investigation is testing the set research question or hypothesis.
- Data can either be qualitative or quantitative.
- Qualitative data is descriptive and unmeasurable and uses descriptions or adjectives to record observations.
- Qualitative data can be nominal (when the order is not important) or ordinal (when the ordering is important).
- Quantitative data is empirically measurable and uses instruments to record observations.
- Quantitative data can be discrete (only able to be recorded as particular numerical values) or continuous (not restricted to particular numerical values, but occurring within a given range).
- Reliability refers to the notion that if the experiment is repeated many times, the results obtained should be consistent.
- Reliability is improved by replication (having multiple samples within an experiment) and repeat trials (repeating the experimental test).
- Risk assessments identify, assess and control hazards.
- HAZCHEM codes are warning symbols used to identify hazardous substances.

KEY QUESTIONS

Retrieval

1 **a** State the meaning of the term 'variable'.

b Copy and complete the table below with definitions of the types of variables.

Independent variable	Measured variables	Dependent variable

2 Name the variables specified in a research question.

Comprehension

3 Write each of the five numbered inferences below as a research question.

a This grass receives the rain run-off from the path when it rains.

b The concrete path insulates the grass roots from the heat and cold.

c People do not walk on this part of the grass.

d The soil under the path remains moist while the other soil dries out.

e More earthworms live under the path than under the open grass.

4 Develop a null hypothesis to test each of the following:

a whether carrot seeds or tomato seeds germinate quicker

b whether sourdough, multigrain or white bread goes mouldy the quickest

c whether Trigg the dog likes dry food or fresh food better

1.8 Review *continued*

5 Identify which of the following pieces of information about plants are qualitative and which are quantitative. Place a tick in the appropriate column.

Information	Qualitative	Quantitative
grow 3.5 mm d^{-1}		
sweet fragrance		
optimum growing temperature 32°C		
leaf length 29 cm		
dark-green appearance		
transpire 80 mL d^{-1}		
fruit taste pleasant		
white sap		

6 Identify the independent, dependent and controlled variables that would be needed to investigate each of the following research questions.

a Will an increase in temperature result in an increase in the rate of transpiration in plants?

b Will increasing the wavelength of light cause no increase of photosynthesis in the leaves of a plant?

c Can a cup of hot chocolate with a lid on it remain at a higher temperature for a longer period of time than a cup without a lid?

d Can the wick of thin candles burn through faster than thick candles?

7 Explain the reasons for having safety data sheets for the chemicals used in the laboratory.

8 Explain the appropriate course of action if you spilled a chemical substance on yourself that carries the following label.

Analysis

9 Identify the difference between quantitative and qualitative data.

10 Select the valid research question and justify why all other options are not valid.

A Can the increase of light and temperature increase the rate of photosynthesis?

B Transpiration is affected by temperature.

C Is light related to the rate of photosynthesis?

D Will mould grow faster on multigrain bread compared to white bread?

11 Consider the following seedling growth investigation.

a State the independent variable for the experiment.

b State the dependent variable for the experiment.

c List the controlled variables stated in the procedure.

d Explain the importance of controlling all variables except the dependent variable.

e Identify three variables that could be used to modify this experiment and describe a modification for each variable.

f Write a research question for each variable used to modify the experiment in question 11e.

g Refine each research question from 11f.

Purpose
To investigate the effect of pH on seedling growth.

Hypothesis
If the soil pH is increased, then seedling growth will increase.

Procedure

1 Germinate twenty pea seeds on damp cotton wool and choose twelve with a height of about 12 mm.

2 Plant a seedling in each of twelve pots of the same size. For each pot, use 80 g of quality potting mix, and water with 10 mL of tap water. Safety note: ensure that gloves and a mask are worn when handling potting mix, as it may contain harmful microbes.

3 Label each pot with the pH treatment the soil will receive: four pots at pH 5, four pots at pH 7 and four pots at pH 9.

4 Weigh each pot to the nearest 0.1 g. Draw up a data table and record the results for each pot in the column for day 0.

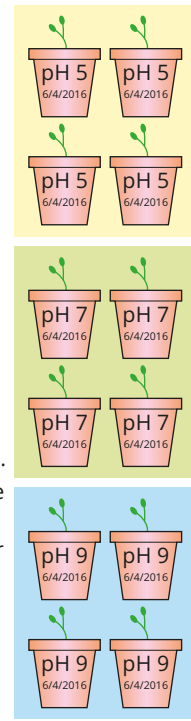
5 Reweigh the seedlings in their pots 2 days later. Record the results for each pot in the column for day 2.

6 Immediately after weighing, give each plant 10 mL of water at the appropriate pH according to the label on the pot.

7 Repeat steps 5 and 6 every 2 days for the next 10 days.

8 Keep plants in the same position where light is available to maintain lighting conditions.

9 Repeat steps 1–8 twice to reduce the chance of variability between trials.



1.9 Conducting an experiment

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- determine relevant data that is needed to test the research question
- determine what is considered to be sufficient data to test the research question
- select appropriate equipment to collect relevant and sufficient data.

Now that you know about the variables involved and have planned your experiment methodology, you can conduct the experiment. Experiments rarely run according to the precise plan.

CONDUCTING AN OPEN-SYSTEM EXPERIMENT

While conducting an open-system experiment, the variables and conditions (for example, temperature, time, date, weather conditions and any other biotic and abiotic factors of influence) must be observed and measured to ensure reproducibility and some level of reliability. Sample size is extremely valuable. Also, remember that the precision of instruments determine the significant figures and together these help the accuracy of the experiment and reliability of the results.

Possible considerations when conducting an open-system experiment

Depending on what your experiment is investigating, there are several aspects of the experiment that should be included in the planning. These include:

- equipment
- instrumentation
- safety precautions
- time (preparation, testing)
- complexity of testing
- chemical concentrations and volumes
- sequential order of activities to complete testing.

Equipment and instrumentation

The choice of equipment and instrumentation will influence the reliability of your experiment. Where possible, use equipment rather than human means to conduct experiments. Some examples are shown in Table 1.9.1 (page e76).

For open-system ecological investigations, recording the sample with a digital photograph is beneficial. It allows repeat data collection or confirmation of sampling at a later date. Large printed images allow other techniques that are not possible in the field (for example, tagging techniques, which ensure improved accuracy with counts). Tagging is usually not possible in the field. With large prints of photo evidence, individuals can be marked (or crossed off) when counted.

When conducting the experiment, use the most precise instruments available to minimise error and lower uncertainty in the measurement. Table 1.9.2 (page e76) demonstrates the benefits of greater precision instruments through lower uncertainty.

TABLE 1.9.1 Comparison of rudimentary and improved experimental testing

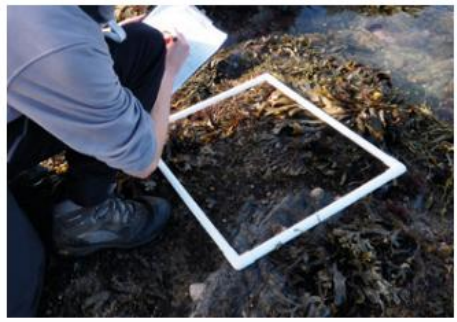
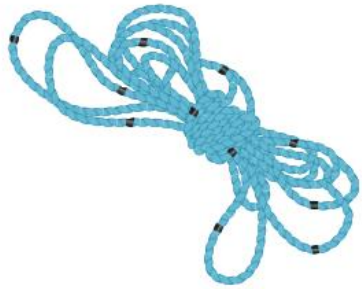
Rudimentary (basic) data collection		
Identifying species using personal notes	Estimating the temperature of water by hand	Conducting a biodiversity study using a basic quadrat 
Improved set-up – accurate and precise data collection		
Identifying species using identification key	Measuring the temperature using a digital thermometer (sensor) with known precision and uncertainty	Conducting a biodiversity study using a segmented quadrat

TABLE 1.9.2 The difference in precision between instruments

Lower precision instrument		
Glass thermometer with a precision of ±2.5°C	Measuring beaker with a precision of ±5 mL	Transect line with 5 m increments 
Higher precision instrument		
Digital laser thermometer with a precision of ±0.2°C	Measuring cylinder with a precision of ±0.25 mL	Transect line with 1 cm increments

Safety precautions

Always use safe procedures. Common sense is essential. For example, all glass equipment and instruments should be used at the back of the bench so other people walking by do not cause an accident. Place a sign on the lab bench warning other students and staff not to touch the equipment. If in the field, set up a work station (even if it is on the ground) so you are not walking around near equipment that is a potential injury hazard, especially glass.

You must follow your school's and teacher's safety and risk assessment guidelines. Completing the risk assessment may require completing a form or completing an online process.

COLLECTING SUFFICIENT AND RELEVANT DATA

It is important to measure and collect the data needed to answer the research question. Collecting sufficient and relevant data is also a characteristic within the research and planning criterion of the student experiment ISMG. Ensure the dependent variable is measured or observed and that every other measurement of data and observation of evidence is directly related to the relationship in the research question or hypothesis. It is recommended that you collect enough data and complete enough sampling to perform statistical analysis and achieve reasonably low uncertainty ranges.

The purpose of scientific experiments is to infer relationships between variables. The relationships must be observed and measured so that understanding can be quantified or an inference established. Understanding can only be achieved through mathematical relationships (known as trends, patterns and relationships) and to achieve this, enough relevant data of all influential variables must be collected.

Collecting sufficient data

The term 'sufficient' is defined by the QCAA Biology syllabus as 'enough or adequate for the purpose'.

Enough data needs to be collected to substantiate whether or not a relationship exists between the independent and dependent variables in the research question. Usually, substantiating a relationship requires statistical analysis. This includes collecting an appropriate number of replicates and also an appropriate number of individual samples.

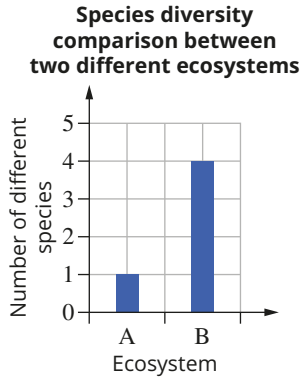
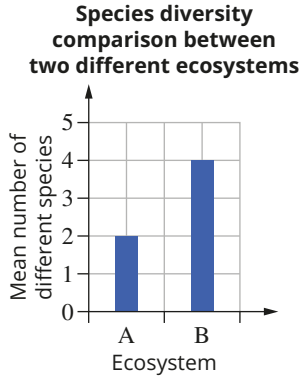
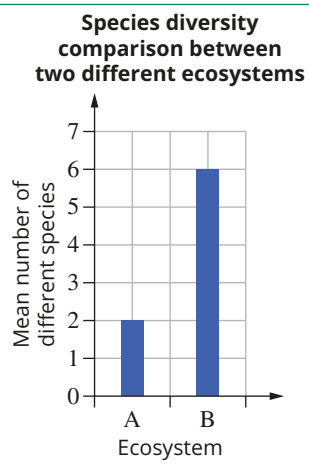
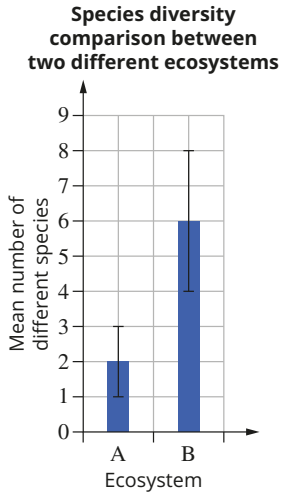
In an open-system biological experiment, replicates cannot be achieved by running the experiment again, because environmental variables that are out of our control change between trials. Replicates are achieved by undertaking the same methodology and collection of data in a separate location. The separate sampling location must be in the same habitat or ecosystem.

The number of replicates will reduce the influence of errors and uncertainty, and improve the reliability of statistical analysis. Errors will always exist, and, in an open-system, an investigation's sample size is one of the only ways to reduce their effect. There should also be enough samples to account for natural variation in a typical population and account for uncertainty. The more individuals sampled and the larger the sample size, the more of the greater population would have been measured. This will result in the statistical analysis (e.g. means) being calculated closer to the true value. Also, unusual measurements and uncertainty will have a lower impact on the quality of data and analysis.

Together, the number of replicates and individual samples determine the sample size. This is vital as it enables a valid interpretation of the data and a valid conclusion to be determined.

Table 1.9.3 (page e78) outlines examples of sample size and their effect on the results of an experiment. The example results are from an experiment that compares the species diversity between two different ecosystems.

TABLE 1.9.3 Examples of sample size and their effect on the results of an experiment

Example of analysed data		Effect of sample size on analysis
The mean number of species in ecosystem B is four times greater than ecosystem A.		Single sample: With only one sample point per ecosystem (1 quadrat each), these results suggest an inappropriate difference in species diversity. This is an unreliable result as no replicates have reproduced the result. This is insufficient data.
The mean number of species in ecosystem B is two times greater than ecosystem A.		Sample of 3 quadrats/ecosystem: The sample size of 3 quadrats (per ecosystem) used to collect this data is insufficient. A mean can be calculated; however, it does not represent the true value of the greater population, and it is not enough data to conduct any other statistical analysis.
The mean number of species in ecosystem B is three times greater than ecosystem A.		Sample of 10 quadrats/ecosystem: The chosen number of individual sample points is sufficient. The calculated mean may represent the true value. True values can only be calculated if the entire population has been measured, which is rarely possible in ecology. Approximately 20 sample points allow statistical calculations (such as mean) to have explanatory power (enough data to make an inference).
The mean number of species for ecosystem B is three times greater than A. The range in standard deviation is clearly different, establishing a difference in natural variation.		Sample of 10 quadrats/ecosystem: Using the same sufficient number of sample points, further analysis has been completed. As only a sample of the entire population has been sampled, the true value (true mean) cannot be known, however, enough data has been collected to establish a significant difference.

Collecting relevant data

The variables to be measured or collected must be directly related to the proposed relationship between the independent and dependent variables. Additional variables can be measured or collected that are indirectly related to the hypothesised relationship, if the background research shows it could be beneficial in the analysis or interpretation of the proposed relationship. If you do not have any background research relating a variable to the research question, then it is not relevant, and therefore is not to be measured or collected.

1.9 Review

SUMMARY

- The choice of equipment and instruments will influence the reliability of the experiment.
- The precision of equipment and instruments is important for accuracy and reliability.
- The collected and measured data must be relevant to the proposed relationship in the research question.
- Equipment used during an experiment should enable you to collect and measure relevant data to address the research question.

KEY QUESTIONS

Retrieval

1 Explain why it is important to choose appropriate equipment and instruments to conduct experiments.

2 Describe sufficient data.

3 Describe relevant data.

Comprehension

4 Explain how the precision of equipment can impact scientific conclusions.

Analysis

5 A student recorded the data below to test the following null hypothesis: Temperature does not affect the rate of germination of radish seeds.

Effect of temperature on seed germination

Temperature (°C)	Light present?	Number of seeds planted	Number of seeds germinated	Seed colour	Seed shape	Colour of germinated leaves
0	Yes	10	0	brown	spherical	x
	No	10	0	brown	spherical	x
10	Yes	10	6	brown	spherical	green
	No	10	5	brown	spherical	yellow

Assess whether or not sufficient and relevant data was collected to address the research question.

1.10 Results

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- analyse raw data to produce processed data
- interpret data to draw valid conclusions.

During the student experiment, you must record all measurements and observations (the journal is helpful for recording the results). This is the raw data. Choosing not to record certain measurements or observations (raw data) is invalid, shows bias and is scientifically fraudulent. Unusual and unexpected measurements and observations may be due to valid relationships between variables that are unknown to the scientist. This cannot be determined until the raw data is processed, analysed and interpreted.

Identifying errors is essential to science. Some can be minimised through careful planning of the methodology while others can be identified through careful analysis. The student experiment ISMG analysis of evidence criterion has characteristics in the performance level descriptors that require the analysis of errors and limitations.

The results, after analysis, need to show whether or not a relationship exists between the variables in the research question. To achieve this, the results need to be presented appropriately. This is dependent on appropriate measurement, observation and recordings (e.g. quantitative or qualitative). Make sure you plan this before conducting the experiment.

Use mathematical and scientific conventions to process the raw data so it can be succinctly represented in tables, graphs, schematics or diagrams. Refer to Module 1.6 for specific guidance on producing quality and appropriate graphs and Module 1.11 for details regarding representing results.

IDENTIFYING ERRORS

Most practical investigations have errors associated with them. As shown in Figure 1.10.1, there are many different types of errors that can occur.

Once data has been systematically tabulated or placed in a graph, some errors can be identified. Some errors may be obvious, such as anomalies. Others may be less obvious and will require statistical analysis. This will help to determine if they are outliers or not.

If the student experiment is not well planned or conducted, there may be numerous errors. It is possible that the number of errors are so numerous that analysis will not find them, as the error values are more frequent than reliable or true values. If this is the case, then the background research and conceptual understanding will help to identify that the results or values are as expected.

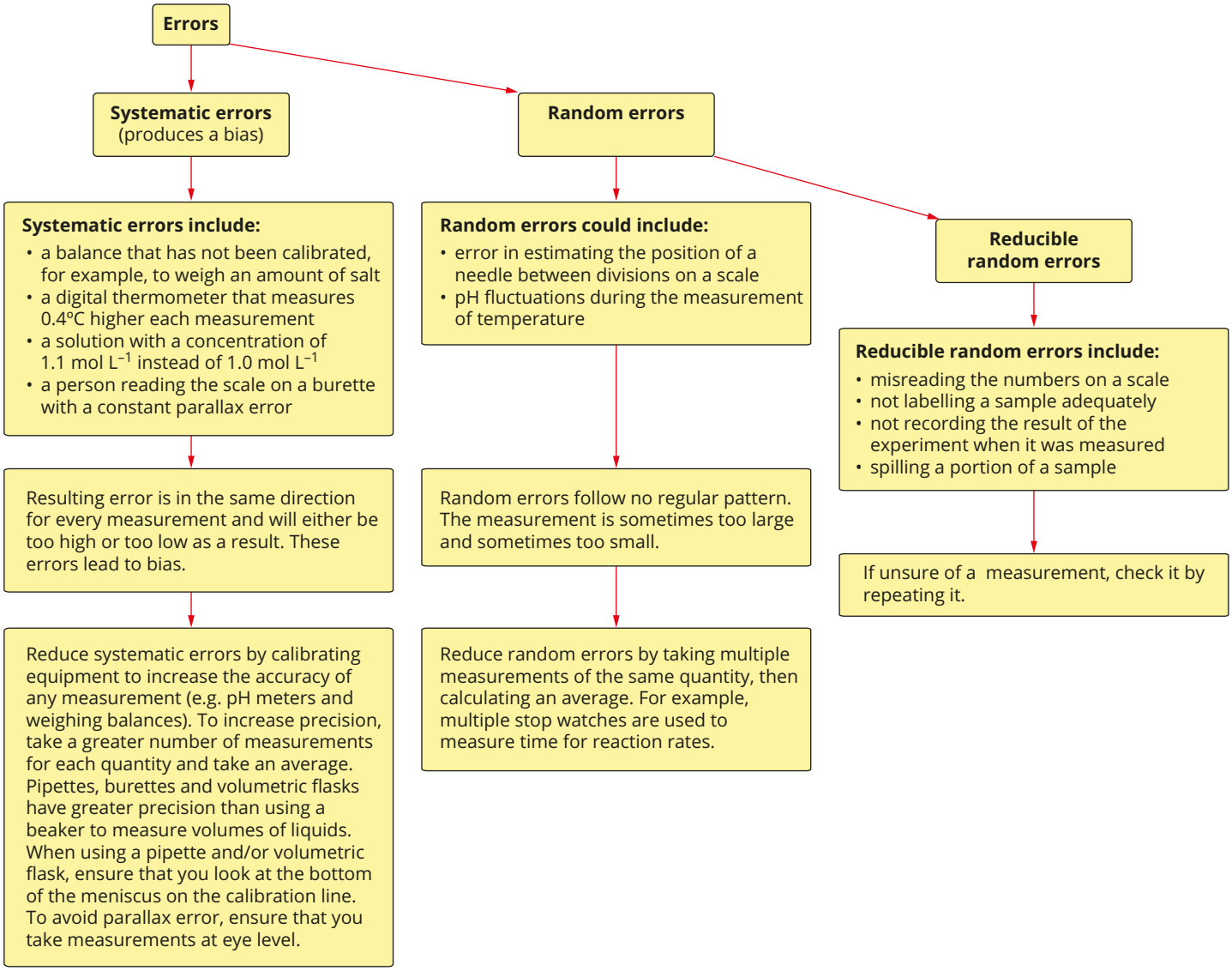


FIGURE 1.10.1 Types of errors that can be made in an experiment.

ANALYSING

Analysing the raw data means searching for relationships between variables, trends or patterns in the data, uncertainties and errors, outliers and results of significance. Analysing raw data produces processed data.

When analysing data, it is important that it is not selectively processed to demonstrate what the scientist wants to see. Such bias will result in using analysis tools (e.g. statistics) inappropriately, erroneously and will result in invalid conclusions and academic fraud (such as false data from the fabrication or alteration of data). Quality scientific analysis processes the raw data as it is and is open to any result.

In this section, you will learn how to discuss your investigation and draw evidence-based conclusions in relation to your research question. This is enhanced by quality presentation of the processed data, including tables, graphs, flow charts and diagrams. See Module 1.6 for more information about presenting data.

Analysing precision

The uncertainty of the instruments determines the precision of measurement, and must always be displayed. They are displayed as a range of data next to the results (e.g. measurement $\pm x$) (Module 1.5). When analysing and processing data with uncertainties remember to apply the appropriate rules (Module 1.5).

It is important to establish whether the variations in results are due to instrument uncertainty or natural variation. Biological variation always exists and is unavoidable in the raw data. The analysis needs to ascertain the source of the variation to enable a conclusion.

If the measurements between samples or tests fall within the uncertainty range of the instrument (Table 1.10.1), then the variation in results could simply be due to the instrument. If the difference between the measured results is greater than the uncertainty range, then the variation in the results is not due to the instrument and is due to other variables (either natural variation or error).

TABLE 1.10.1 Size of red waratah anemones in the lower-littoral zone

Sample	Diameter (mm)
organism 1	33 ± 0.5
organism 2	39 ± 0.5
organism 3	28 ± 0.5
organism 4	42 ± 0.5
organism 5	45 ± 0.5
organism 6	28 ± 0.5
organism 7	29 ± 0.5
organism 8	41 ± 0.5
organism 9	35 ± 0.5
organism 10	28 ± 0.5
mean	35 ± 0.5

Table 1.10.1 shows the size of red waratah anemones measured in a quadrat in the lower-littoral zone. The uncertainty in the measurement is determined by the instrument and is ±0.5 mm (it is precise within ±0.5 mm). As the difference between organism 6 and 7 is within the uncertainty of the instrument, it cannot be determined whether the difference in the measurements is due to natural variation or instrumental precision. It must be interpreted that there is no biological difference between organism 6 and 7.

The difference between organism 5 and 6 is greater than the instrumental precision (uncertainty), therefore the difference is due to reasons other than the instrument. If the methodology controlled the **extraneous variables** appropriately and nothing unforeseen influenced the results, then the difference between the organisms is interpreted to be due to biological reasons.

Analysing validity and theoretical relationships

Some of the first processes in analysing data is to determine the true values (with uncertainties) and measures of central tendency (if possible). The evidence can then be processed to find errors in measurements.

Once the quality of the data is understood, the validity in relation to established theoretical concepts can be analysed. This can be achieved by performing statistical analysis to produce a reliable description of relationships or determine significance in the data or between variables. Eventually the analysis will provide evidence to answer the research question. Here is a small and non-exhaustive list of calculations that can be used to analyse relationships.

- Pearson correlation coefficient: determines if a linear relationship exists (that the variables correlate), the direction and how strong the linear relationship is (refer to page e36 of *Pearson Biology 11 Queensland* for further information)
- Coefficient of determination: estimates how much of the results can be predicted due to the change in the independent variable (refer to page e37 of *Pearson Biology 11 Queensland* for further information)
- Spearman's rank correlation: determines if a non-linear relationship exists in a single direction (refer to page e41 of *Pearson Biology 11 Queensland* for further information)
- student *t*-test: determines if there is a difference between two means beyond natural variation (normal distribution) (refer to page e41 of *Pearson Biology 11 Queensland* for further information).

Example analysis of evidence

Table 1.10.2 shows the size of red waratah anemones at two different locations in the lower-littoral zone where there is a difference in species diversity. The difference in mean diameter (mm) of red waratah anemones between location one (quadrat 1 with a Simpson's diversity index of 0.21) and location two (quadrat 2 with a Simpson's diversity index of 0.55) is 2.0 mm. This is greater than the instrumental uncertainty and therefore is due to biological differences, if methodological issues or unforeseen variables can be excluded. Since the difference in results has been established to be due to experimental variables and not the instrument, processing the data using statistics can analyse and help ascertain which variable is the cause of the difference.

It is important to choose the appropriate statistical calculation to analyse the data. Scientists refer to resources to help choose the appropriate statistical analysis. Module 1.5 outlines many useful statistical calculations commonly used in biology. Refer to this section every time you are processing the results of your class practicals or student experiment.

An appropriate analysis may ascertain if there is any validity in the data or if there are true biological differences or relationships. Without any further statistical calculations, there are a number of plausible explanations for the results in Table 1.10.2. Below is a list of some examples:

- sample bias—the selection criteria used to choose the locations may have caused systematic error
- error in methodology—the technique for using the calipers may have been inconsistent, causing random or systematic error
- error in methodology—the technique to count the number of organisms for each species may have been changed between locations, possibly resulting in some organisms to be measured twice, causing random error
- there could be an error in the calculation of the Simpson's diversity index, or collecting data for the species diversity index
- the amount of exposure to submersion or direct sunlight may be vastly different, an abiotic factor rather than biotic and an extraneous variable.

In the example shown in Table 1.10.2, if all variables were controlled and observations made during the experiment confirmed the experiment went to plan (minimal systematic errors), then statistical processing can investigate differences. In this case, the *t*-value can be calculated as shown in Table 1.10.3, which calculates the likelihood that the difference between the means is due to natural variation rather than the independent variable (Simpson's diversity index).

Table 1.10.3 shows the *t*-value, 0.48, for the comparison of the two red waratah diameter means. This means that there is a 48% chance that the difference in the means is due to natural or biological variation. This is greater than the threshold for significance of the student *t*-test which is 0.05, and therefore the difference is *insignificant*. The species diversity, the independent variable, is interpreted to *not* have a significant effect on the size of red waratah anemones.

In this example, if the background research revealed that no relationship had been established between these two variables, then the analysed results could be interpreted to support the theory. Then an analysis could be performed to search for random and systematic errors, specifically anomalies and outliers that may have affected the results.

Finding anomalies and outliers

Statistical analysis can find anomalies and outliers in data that are not valid measurements. Most importantly, during the student experiment, your record of observations may provide a reason for any outliers in your data. This can then be used to explain invalid or unexpected results and may also suggest improvements in the methodology to remove such measurements.

TABLE 1.10.2 Comparison of red waratah anemone size in the lower-littoral zone in different locations with different species diversity

Sample	Diameter (mm) Quadrat 1 (Simpson's diversity index: 0.21)	Diameter (mm) Quadrat 2 (Simpson's diversity index: 0.55)
organism 1	33 ± 0.5	40 ± 0.5
organism 2	39 ± 0.5	31 ± 0.5
organism 3	28 ± 0.5	41 ± 0.5
organism 4	42 ± 0.5	25 ± 0.5
organism 5	45 ± 0.5	29 ± 0.5
organism 6	28 ± 0.5	30 ± 0.5
organism 7	29 ± 0.5	31 ± 0.5
organism 8	41 ± 0.5	36 ± 0.5
organism 9	35 ± 0.5	35 ± 0.5
organism 10	28 ± 0.5	31 ± 0.5
mean	35 ± 0.5	33 ± 0.5

TABLE 1.10.3 Comparison of red waratah anemone size in the lower-littoral zone in different locations with different species diversity

Sample	Diameter (mm) Quadrat 1 (Simpson's diversity index: 0.21)	Diameter (mm) Quadrat 2 (Simpson's diversity index: 0.55)
organism 1	33 ± 0.5	40 ± 0.5
organism 2	39 ± 0.5	31 ± 0.5
organism 3	28 ± 0.5	41 ± 0.5
organism 4	42 ± 0.5	25 ± 0.5
organism 5	45 ± 0.5	29 ± 0.5
organism 6	28 ± 0.5	30 ± 0.5
organism 7	29 ± 0.5	31 ± 0.5
organism 8	41 ± 0.5	36 ± 0.5
organism 9	35 ± 0.5	35 ± 0.5
organism 10	28 ± 0.5	31 ± 0.5
mean	35 ± 0.5	33 ± 0.5
t-value	0.48	

It is important to note that statistical calculations cannot differentiate the source of the data. They are just numbers. Care must be taken to use statistical calculations appropriately. Analysing data is a combination of processing numbers and the experimenter placing those numbers into a context. It is important the researcher knows what the numbers are, how they were measured and their meaning, and knows which statistical calculation to use to understand mathematical relationships that may represent biological relationships. It is also vitally important to record observations during the experiment so that the correct context can be given to the processed data.

Anomalies

An anomaly is a value that seems to deviate from the typical or expected outcome (relationship, trend or pattern). It may be possible for a researcher to find an anomaly by viewing the data without using statistical analysis. Figure 1.10.2 is an example of an anomaly: the value of 8.6 (y-axis) at 6 (x-axis) deviates from the trend. Anomalies can also be statistically determined: they are values (or measurements) that have deviated from the expectation without a known reason. A statistically determined anomaly provides a stronger argument about its deviation from the expected, as the statistical calculation will provide a value as to how much deviation has occurred. This calculated value of deviation also provides justification for suggesting improvements to the methodology.

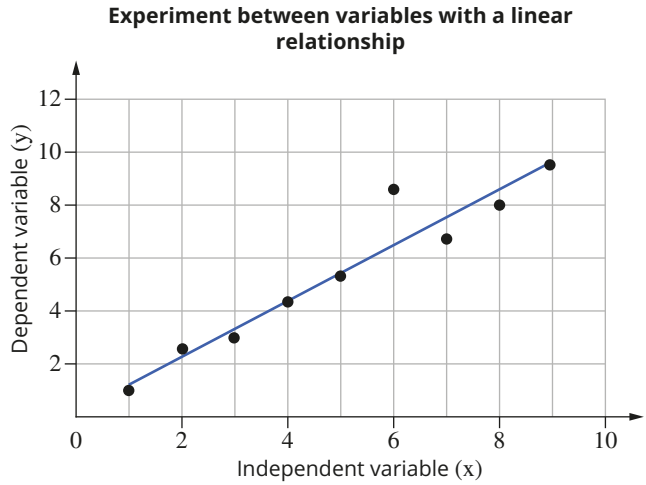


FIGURE 1.10.2 The anomaly is the data point that obviously deviates from the trend.

Outliers

An outlier cannot be determined by the researcher simply on the appearance of the value. An analysis needs to be done to establish that a value is significantly different and to establish that it lies outside the expected. The analysis needs to establish the distance the numerical value is from the expectation. This distance must be outside an acceptable range (e.g. standard deviation of 1σ, 2σ or 3σ; interquartile range; standard error) to be determined as an outlier.

Table 1.10.4 illustrates the display of an outlier in a graph. Trial 6, test 1 has been identified as an outlier. It is a data point that lies outside the expected. In this case the measurements were expected to be within 2σ (2 standard deviations) of the mean. Displaying outliers helps to explain larger ranges (e.g. range or standard deviation) in some measurements, especially when the measured range was unexpected. It may help to explain why data did not follow the expected trend closely. It can also provide justification for suggested improvements to the investigation or experiment.

TABLE 1.10.4 Data displaying an outlier

Trial	Test 1	Test 2
1	5.5	7.2
2	5.6	7.3
3	6.1	7.1
4	5.9	7.5
5	6.0	7.7
6	6.6	7.4
7	5.9	7.4
8	5.8	7.4
9	5.9	7.1
10	6.0	7.2
mean	5.9	7.3
standard deviation	0.3	0.2

INTERPRETING

Once the results have been processed and analysed, scientists offer explanations of what occurred and a possible reason why. Processing and analysing data is the manipulation of the numbers or observations to understand and ascertain true values, uncertainties, errors, anomalies and relationships. Interpreting is placing this understanding into words and providing an in-depth explanation of what the numbers mean. It is writing an explanation of the results.

Interpretations should never provide an explanation beyond the constraints of the experimentation and methodology. Interpretations are not meant to provide all the answers or comprehensive explanations for everything related to an experiment. Interpretations are only valid and reliable if they are based on what was measured. It is important to note that science can never measure the true value of a phenomenon. Therefore, the interpretation is always an inference. The interpretation of results in the scientific report should always be concise.

It is important to interpret the measured results and not the planned or expected results.

- If the results follow expectations then this research can be used to interpret the theoretical reasons for the measured results. However, ensure the interpretation stays within the limits of the uncertainties, instrumental precision and statistical analysis (e.g. r-values or R^2 values, student t-test). For example, if you have not determined statistical significance, do not state that the results are significant. If an outlier has not been determined by statistical calculations, do not state a result or measurement is an outlier.
- When the results do not follow expectations, interpret them as such. You still need to relate the results to theory, and so you may need to do further research in order to explain the results. This is where recording observations during the experiment in the journal becomes invaluable. When the results do not occur as expected, the observation provides a clue or a basis for what concepts or relationships need to be further researched. Further research will provide the theory to offer a plausibility (infer a reason) for the results. If the results are not as expected, it is important that you use statistical analysis to establish that the results did not follow expectations, rather than just stating because it 'looks' like it.

Once the interpretation is complete in your journal, you can write the scientific report, summarising all the scientific work and method used for the experiment.

1.10 Review

SUMMARY

- Raw data includes the measurements and observations made during an experiment.
- Processed data is derived from processing and manipulating raw data.
- Processed data enables trends, patterns and differences to be identified.
- If the measurements between samples or tests fall within the uncertainty range of the instrument, then the variation in results could simply be due to the instrument.
- Interpretations of data attempt to explain the observed results.

KEY QUESTIONS

Retrieval

1 State the two types of data.

2 Define systematic error.

3 Describe random errors.

Comprehension

4 Use a graphic organiser to summarise what is involved in each of the following:

a processing of data

b interpreting data

Analysis

5 The atmospheric temperature in a dry sclerophyll forest was measured at the ground level (0.1 m) and the average height of the first level of above-ground foliage (1.1 m). Analyse the data below to determine if it can be concluded that there is a temperature difference.

Atmospheric temperature in a dry sclerophyll forest at ground level (0.1 m)

Sample	Temperature 0.1 m above ground (°C)	Temperature 1.1 m above ground (°C)
quadrat 1	22.1 ± 0.05	20.2 ± 0.05
quadrat 2	22.5 ± 0.05	23.5 ± 0.05
quadrat 3	21.9 ± 0.05	21.7 ± 0.05
quadrat 4	24.5 ± 0.05	24.6 ± 0.05
quadrat 5	23.6 ± 0.05	24.1 ± 0.05
quadrat 6	22.0 ± 0.05	23.5 ± 0.05
mean	22.8 ± 0.05	22.9 ± 0.05

6 The mean Simpson's diversity index (SDI) was calculated for two different habitats within an ecosystem. Analyse the following data to determine if it can be concluded that there is a difference in the biological diversity between the two habitats.

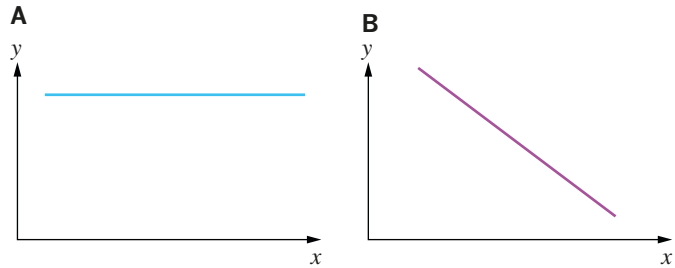
Mean SDI for habitats A and B

Sample	Habitat A (SDI)	Habitat B (SDI)
quadrat 1	4	6
quadrat 2	3	3
quadrat 3	2	2
quadrat 4	5	5
quadrat 5	2	8
quadrat 6	3	6
quadrat 7	3	4
quadrat 8	2	7
quadrat 9	2	4
quadrat 10	4	1
mean	3	5
standard deviation	1	2
t-value	0.05	

7 **a** Distinguish the graph below that is displaying a change in transpiration as temperature changes.

b Distinguish the graph that illustrates the following observation:

You are growing a bacterial culture on agar and make observations from days 3 to 6 in which the results seem to indicate a plateau in the number of colonies.



1.11 Communicating and writing a scientific report

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- identify and explain the sections of a scientific report
- write a scientific report.

To write a scientific report, you need to follow some general conventions. Even though there are many ways to present a report for the student experiment, it must follow a scientific genre and meet the requirements of the QCAA Biology syllabus. This module will provide a guide to writing an appropriate scientific report.

> Your journal should contain all the information required to complete your scientific report for the student experiment. After all, your scientific report like any other report summarises research and information gathered on a topic. The scientific report does not require much time to complete when reporting from a comprehensive journal.

SCIENTIFIC WRITING AND LITERACY

Scientists use words (the agreed scientific language and terms) that have specific meanings, which may differ from the understanding of the term in common language. This allows a common understanding of words across all languages, to convey scientific meaning. Table 1.11.1 provides some examples of the differences in the understanding of words in common language and in scientific language (even if it is slight).

TABLE 1.11.1 A comparison of common and scientific language

Term	Common language meaning	Scientific language meaning	Difference in meaning
sample	A representative part or a single item from a larger whole or group, especially when presented for inspection or shown as evidence of quality.	The specifically chosen physical representatives of a phenomenon, that was tested, measured or observed during experimentation or an investigation.	Very similar. The scientific term is specific to a controlled or measured phenomenon. In common language, 'sample' can be used generically while in scientific language it is specific to the independent variable.
results	To proceed or arise as a consequence, effect or conclusion; to have an issue or result.	The recorded evidence of the sample during experimentation or observation.	Very similar. The scientific term requires measurement or explicit recording of the observed consequence or effect arising from the independent variable.
significance	Something that is conveyed as a meaning often obscurely or indirectly; the quality of conveying or implying; the quality of being important.	Important; of consequence; expressing a meaning; indicative; includes all that is important; sufficiently great or important to be worthy of attention; noteworthy; having a particular meaning; indicative of something.	Similar. The scientific term refers to the quality of being important. To establish importance, statistical analysis has to have been conducted to achieve the meaning, or indicate sufficient difference, or worth, and to indicate a particular meaning.
correlation	The state or relation of being correlated; specifically, a relationship existing between phenomena or things or between mathematical or statistical variables that tend to vary, be associated or occur together in a way not expected on the basis of chance alone.	A relationship existing between phenomena on the foundation of statistical or processed analysis.	Almost identical. The scientific term refers to when data processing or statistics has been applied to the common term. As such, the strength of the correlation can be predicted or assumed.

Ensure that the report is written using scientific language and conventions. Your report should be fluent and concise. Carefully plan all parts of the report, including:

- introduction
- summary of altered methodology
- results
- analysis and interpretation
- evaluation and conclusion
- suggested improvements.

Writing fluently and concisely

The report should be brief and comprehensive, so only use the words required to communicate your information. Writing a concise report avoids repetition and helps you remain within the required word count or length. Use scientific language as it allows you to communicate details and knowledge in fewer words. Table 1.11.2 demonstrates communication of the same concept in a few different ways. Being fluent and concise will significantly influence the word count of your report and the quality of your writing.

TABLE 1.11.2 Communicating concepts in various ways

Concept	Communication	Commentary of communication
outlining the basic details of a virus	Viruses are small molecules that use cells to reproduce.	This is fluent and concise. However, it lacks scientific terms or language to convey in-depth understanding.
	Viruses are tiny little objects made up of numerous parts that infect cells. They cannot reproduce themselves so they need other cells to replicate them, using their own (host cell) components. Inside a virus, you will find genetic molecules such as DNA and RNA. These are surrounded by a capsule or envelope called a capsid. Therefore, viruses are tiny and need other cells to replicate/reproduce.	This is fluent, but not concise. There is repetition (e.g. 'tiny' and 'little', which have the same meaning, and 'reproduce', which is stated twice). It also uses many words without using scientific terms to provide detail and understanding. This may be useful language in the journal when learning about a topic, but not in the scientific report.
	Viruses are microscopic obligate, intracellular parasites. They are composed of genetic material enclosed in a capsid and cannot replicate without the host cellular components.	This is fluent and concise. Much in-depth understanding is communicated through scientific terms, which enables quality information to be conveyed in fewer words.

Writing in a scientific genre

There are a number of genres or styles of scientific communication, including literature reviews, empirical essays and poster presentations. This module will focus on scientific reports. A report is a document that communicates a summary of information, focusing on the main points of interest. It uses headings, sections, tables and graphs to present information. A scientific report is written to scientific conventions, including format and language.

Use of headings

Using headings in scientific reports is essential. There are international conventions for scientific report writing; however, they are specific to the journal publication. There is no single convention for scientific report writing. Table 1.11.3 lists headings that are commonly used in scientific reports and describes the information that would be provided under each heading. Sections can be broken down further into subsections. Some subheadings are suitable for more than one section; however, each scientific report will only use each heading and subheading once. It is best to ask your teacher about the headings they prefer and how to align your scientific report and its headings to the syllabus.

TABLE 1.11.3 Scientific report sections, headings and information

Scientific report sections	Common title alternatives	Expected information within the section
Title	–	A specific statement that outlines the expected relationship between independent and dependent variable. It can also be a question asking about the relationship between the independent and dependent variable.
Abstract	–	A summary of the entire experiment/investigation in a single paragraph outlining the main information for each section: background information, method, results, analysis and conclusion. It usually includes 1–3 sentences per section and less rather than more is preferable.
Introduction	• Background information • Background research • Literature review • Rationale	Information already known or inferred from previous experimentation and scientific literature specific to the research question that can explain the reason for the experiment. This information is used to rationalise the experiment. Specifically, scientific knowledge is used and applied to the relationship in the research question between the independent and dependent variable, and any other variables considered by the methodology that may alter the relationship.
Method: • sampling technique • preparation • experiment	• Procedure • Modifications to methodology	An outline of the exact details, including specific details about instruments, equipment models and precision, techniques employed and all information so other scientists can repeat the experiment. It is not common to differentiate the materials (equipment and instruments used) from the procedure that uses them.
Results: • raw data • analysis • statistical analysis • interpretation	• Processed data • Analysis • Interpretation	The type of data presented is unique to specific journal publications. However, in general, all the data, observations and results need to be presented to explain the interpretation and conclusion. The results must show all the required information to answer the research question.
Conclusion: • analysis • interpretation • discussion • evaluation	• Interpretation • Discussion • Evaluation • Sources of error • Suggestion for improvements/ modifications	An explanation of the results, including quality of experimentation (accuracy, precision, validity and reliability of the methodology), relating the results to current scientific understanding (theory). It also considers the strength of the relationship between the experimental evidence (data, observations or results).
References	–	A list of all sources used in the scientific report.

Scientific writing

Because the report is written for a scientific audience, it contrasts with English writing used in everyday situations (for example, scientific language and conventions).

The student experiment report is to be written using paragraphs, with each paragraph explaining only one idea. The first sentence (topic sentence) of the paragraph introduces the topic. The following sentences provide the details of the idea. The final sentence concludes the idea. Each sentence should flow onto the next, slowly building the details of the explanation. The report should be brief and comprehensive. Only use the words required to communicate, and use language that conveys detailed understanding.

Reports should be written in:

- past tense, because the experiment was conducted in the past
- third person, passive voice and impersonal verbs—science uses this language convention (Table 1.11.4 on page e90)
- scientific language—the terms used are specific to concepts, models and theories
- objective, unbiased language—avoid subjective and emotional or persuasive writing (Table 1.11.5 on page e90)
- concise language—avoid unnecessary repetition and express ideas.

Scientific language allows more details, knowledge and understanding to be communicated in fewer words. Table 1.11.6 (page e90) shows some examples of concise wording.

TABLE 1.11.4 Examples of first-person versus third-person narrative

First person	Third person
I put 50 g of marble chips in a conical flask and then added 10 mL of 2 mol L^{-1} hydrochloric acid.	First, 50 g of marble chips were weighed and then placed into the conical flask, then 10 mL of 2 mol L^{-1} hydrochloric acid was added.
After I observed the reaction, I found that ...	After the reaction was completed, the results showed ...
My colleagues and I found ...	Researchers found ...

TABLE 1.11.6 Examples of wordy versus concise language

Wordy language	Concise example
Due to the fact that ...	... because ...
Smith undertook an investigation into ...	Smith investigated ...
It is possible that the cause could be ...	The cause may be ...
End result ...	Result ...
In the event that ...	If ...
Shorter in length ...	Shorter ...

TABLE 1.11.5 Examples of persuasive writing versus scientific writing styles

Persuasive writing	Scientific writing
Use of biased and subjective language • The results are extremely bad, atrocious, wonderful ... • This is terrible because ... • This produced a disgusting odour. • Health crisis	Use of unbiased and objective language • The results showed ... • The implications of these results suggest ... • The results imply ... • This produced a pungent odour. • Health issue
Use of exaggeration • The object weighed a colossal amount, like an elephant.	Use of non-emotive language • The object weighed 256 kg.
Use of everyday or colloquial language • The bacteria passed away. • The results don't ... • The researchers had a sneaking suspicion ...	Use of formal language • The bacteria died. • The results do not ... • The researchers predicted/hypothesised/theorised ...

Presenting scientific ideas

An efficient way of presenting complex data and explaining scientific concepts is through photographs, graphs, tables and scientific models such as flow charts and diagrams. Ensure you include:

- a descriptive title
- labels, captions or descriptions, units of measurement, uncertainty
- numbering, for example, Figure 1, Figure 2, Table 1, Table 2
- a source if the work is not your own or is adapted from work that is not your own.

Using tables and graphs

In general, tables provide more detailed data than graphs. However, it is easier to observe trends and patterns in graphs, making them a very useful tool for presenting evidence. Pie charts illustrate percentages well, while bar charts and line graphs illustrate relationships between variables well. Tables and graphs will communicate in-depth information concisely. More information on tables and graphs can be found in Module 1.6.

Editing your report

Editing your report is an important part of the process. After editing your report, save new drafts with a different file name and always back up your files in another location. Pretend you are reading your report for the first time when editing. Once you have completed a draft, it is always good practice to read your work a few hours or a day or two after you have completed it. Reading your work out loud helps you pick up errors.

When editing, look for content that:

- is ambiguous or unclear
- is repetitive
- is awkwardly phrased
- is too lengthy
- is not relevant to your research question
- is poorly structured
- lacks evidence
- lacks a reference (if it is another researcher's work)
- contains spelling mistakes.

Acknowledging sources

The student experiment does not require a bibliography. A **reference list** is sufficient. A bibliography is a list of all the sources you used during the research to develop understanding (including information in the journal), even if the information was not used directly or explicitly in the scientific report. A reference list only lists the sources used and cited (or referenced in the text) in the scientific report.

All the quotations, documents, publications and ideas used in your scientific report need to be listed in the references and acknowledgements. In order to avoid plagiarism and ensure you properly credit other people for their work, you must complete this accurately. References and acknowledgements also give credibility to your study and allow the reader to locate information sources if they wish to study the topic further.

Plagiarism is using other people's work without acknowledging them as the author or creator. To avoid plagiarism, include a reference every time you use the work and information from others, for example, at the end of a sentence or following a diagram. If you quote directly from a source, enclose it in quotation marks. This will ensure you credit the original author and will enable the reader to find the original source.

Reference list

List your sources at the end of the report in alphabetical order (by the author's last name or the organisation name).

Compile your references in a separate document as you conduct the student experiment. The APA (American Psychological Association) style is currently the most commonly used referencing style.

In-text citations

Each time you write about the findings of other people or organisations, you need to include an in-text citation within the sentence. Provide the full details of the in-text cited source in a reference list. In the APA style, in-text citations include the first author's last name and date in brackets (author, date).

The following examples show the use of an in-text citation and the associated reference list entry.

- In-text: It was reported that in testing of five pro-oxidant additives added to commonly manufactured polymers, none resulted in significant biodegradation after three years (Selke et al., 2015).
- In-text: Selke et al. (2015) reported that in testing of five pro-oxidant additives added to commonly manufactured polymers, none resulted in significant biodegradation after three years.
- Reference list: Selke, S., Auras, R., Nguyen, T. A., Aguirre, E. C., Cheruvathur, R., & Liu, Y. (2015). Evaluation of biodegradation—promoting additives for plastics. *Environmental Science & Technology,* 49(6), 3769–3777.

ADDRESSING THE SYLLABUS REQUIREMENTS

Your final scientific report must address all the characteristics in the performance level descriptors of the student experiment ISMG. Before you begin the scientific report, plan the sections and titles and then assign the ISMG characteristics to each section. As outlined earlier, there is no single convention for the scientific report, and the student experiment ISMG characteristics will fit into any scientific report style (Table 1.11.3 on page e89). You need to decide where to include the ISMG characteristics, after discussion with your teacher.

Word limits or word count guides can be assigned to each section. Apply a larger word count to the sections requiring explanations such as introduction, discussion/conclusion and perhaps the analysis (depending on the convention chosen). This word allocation will act as a guide, but is flexible. Do not exceed the word limit outlined in the ISMG.

When you edit your completed report, assess whether it includes all the evidence in the four criteria (research and planning, analysis of evidence, interpretation and evaluation, communication) that make up the ISMG (IA2). These are the words that follow 'demonstrated by …'.

1.11 Review

SUMMARY

- A scientific report commonly has the following features.
  - Title: A specific statement that outlines the expected relationship between independent and dependent variable.
  - Abstract: A single-paragraph summary of the entire experiment/investigation.
  - Introduction: An explanation of the current scientific knowledge about the relationship between the independent and dependent variable (demonstrating application of understanding) and any other variables that may alter the relationship (considered rationale).
  - Method: An outline of the exact details of the experiment, including specific details about instruments, equipment models and precision, techniques employed and all information required for others to repeat the same results. This should include both the original practical and modified elements of the experiment. Details of the methodology must enable sufficient data to be collected.
  - Results: The relevant data, observations and results relating to the experiment.
  - Conclusion: An explanation of the results, including quality of experimentation. The explanation is related to current scientific understanding. The strength of the relationship between the experimental evidence is stated.
  - References: A list of all sources used in the scientific report.
- The scientific report must address the requirements of the syllabus.

KEY QUESTIONS

Retrieval

1 Describe the information that is included in the following sections of a scientific report:
- **a** method
- **b** conclusion

2 Recall in which section of a scientific report you would find processed data.

Comprehension

3 Explain when it is appropriate to use a:
- **a** bibliography
- **b** reference list

Analysis

4 A scientist designed and conducted an experiment to test the following research question: Could an increased consumption of fast food cause a decrease in the function of the liver in people?

- **a** The discussion section of the scientist's report included comments on the accuracy, precision, reliability and validity of the investigation. Read each of the following statements and identify which category they belong to: precision, reliability or validity.
  - **i** Only teenage boys were tested.
  - **ii** Six boys were tested.
- **b** The scientist then extended the fast food study with 50 teenage boys in the experimental group and 50 teenage boys in the control group. In the experimental group, all 50 subjects gained body mass. The scientist concluded that all the subjects gained weight as a result of the experiment. Deduce whether or not this conclusion is valid.
- **c** Provide recommendations to improve the reliability of this investigation.

PART C RESEARCH INVESTIGATION (IA3)

The QCAA stipulates that students complete a research investigation (IA3) in Unit 4 Biology. The research investigation assessment requires students to investigate a claim. Students must draw on secondary evidence from credible scientific sources such as scientific journals, books, government websites, universities, independent research bodies or science and technology manufacturers. Research conventions are to be used to analyse and interpret the evidence in order to reach a justifiable conclusion about the claim.

The QCAA Biology syllabus states that students must work individually to develop and investigate their own research question based on a number of possible claims the teacher provides.

The research investigation constitutes 20% of the total assessment.

A summary of the research investigation (ISMG IA3) is provided below. The summary includes the objectives, specifications and marking for this summative internal assessment. The research investigation may be presented in:

- written form (e.g. scientific report) of 1500–2000 words, or
- multimodal presentation form (e.g. poster presentation) of 9–11 minutes.

Criteria	Assessment objectives	Demonstrated by:	Marks
research and planning	• apply understanding • perform an investigation	• a carefully and deliberately constructed rationale identifying an easy-to-understand development of the claim from the research question • a research question that is clear, applicable and pertinent to the methodology • selection of adequate, applicable and pertinent resources	6
analysis and interpretation	• analyse the evidence sourced during the research • interpret research evidence	• identification of adequate, applicable and pertinent research question • identification of complete and pertinent trends, patterns and relationships, including every detail • identification of limitations of evidence with careful attention to detail and is fitting to the research question and methodology • reasoned scientific arguments based on evidence	6
conclusion and evaluation	• interpret the evidence from the research • evaluate the processes, claims and conclusions within the research	• reasoned conclusion directly related to the research question • insightful examination by argument of the quality of evidence • extrapolation of worthy and convincing results of the research to the claim • suggested modifications to the investigation that are carefully and deliberately chosen as pertinent to the claim	6
communication	• present the research findings, including arguments and conclusions	• using scientific language and representations that are easy to read, eloquent, clear and succinct • acceptable and suitable use of genre conventions • use of appropriate referencing conventions	2
Total			20

The scientific inquiry is not a linear process. Scientists will not necessarily complete these steps in the stated order and some steps may need to be repeated or altered in order to more accurately address the research question.

Instrument-specific marking guide

Student responses are assessed against an instrument-specific marking guide (ISMG). In developing your research investigation and planning your response, it is important to always have in mind the assessment objectives, in particular, the characteristics that are described in the performance level descriptors.

The major features of the ISMG are outlined below and shown for the research and planning criterion. Just as with the student experiment, the ISMG is organised into:

- four criteria, though these differ for the research investigation—research and planning, analysis and interpretation, conclusion and evaluation, and communication
- performance levels, against which the qualities of the response are assessed
- performance level mark, which may be a single mark or two-mark range
- performance level descriptor.

Criterion: Research and planning

Objectives

These are the objectives being assessed. Your work must demonstrate these objectives. Definitions of the objectives can be found in the syllabus glossary. How the objectives will be assessed is described below.

Descriptors

Each of the dot points in each performance level descriptor is called a descriptor. The descriptors contain all the characteristics required to achieve that level of performance. They outline the evidence that teachers will search for in your work.

Performance level descriptor

The performance level descriptor is the whole of the left-hand cell in each performance level and is made up of all the descriptors in that level.

Objectives of assessment task

2 apply understanding of … to develop research questions
5 investigate … through research

Key features that distinguish between marking levels:	Marks
• Applying an understanding of the subject matter in an informed way that demonstrates development of the research question from the claim • Applying an understanding of the subject matter in an informed way, that demonstrates careful consideration of the rationale for the question • Development of a research question that is specific and relevant from effective and efficient investigations • Using sufficient resources that are relevant in an effective and efficient investigation	5–6
• Applying an understanding of the subject matter in an adequate way that demonstrates links from the claim to the research question • Applying an understanding of the subject matter in an adequate way that demonstrates a reasonable rationale for the question • Development of a research question that is relevant from an effective investigation • Using resources that are relevant to an effective investigation	3–4
• Applying an understanding of the subject matter in a rudimentary way that demonstrates a weak rationale between the research question and the claim • Development of an inappropriate research question from an ineffective investigation • Using irrelevant and insufficient resources in an ineffective investigation	1–2
• Descriptors not addressed	0

Performance level

Together, the performance level descriptor and the marks are known as the performance level. Once the most appropriate performance level has been identified (i.e. the one where most descriptors are matched) then your response can only receive one of the marks in the corresponding performance mark column. If all the descriptors of the performance level are demonstrated in your response, then the upper mark is awarded. Otherwise, the lower mark is awarded.

Performance mark

The mark awarded to the response for that criterion. It is related to the quality of the response as measured against the performance level descriptors.

The following modules outline the steps that assist the development of the research investigation report.

1.12 Developing the research question from a claim

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- analyse a claim to identify scientific concepts, variables and measurable terms within a claim
- develop a research question from a claim.

The research question should specifically address one of the concepts associated with the claim. It should clearly state the relevant variables. All the research conducted for the research investigation will be directly related to the research question. Therefore, the process begins with the claim and understanding the concepts it addresses.

UNDERSTANDING THE CLAIM

The QCAA Biology syllabus defines a **claim** as 'an assertion made without any accompanying evidence to support it'.

The assertion or claim can be a sentence, a statement within a sentence, the title of an article, a quote or anything published in any form. The assertion has to be in isolation from any justification using data, research, evidence or reason from known information to support it (Figure 1.12.1). Your teacher will provide you with a number of possible claims. Your task will be to develop a research question based on one of these claims. Your research question will focus your investigation, necessitating evidence to be gathered so that the claim can be evaluated.

How is a research question formed from a claim that is not supported by evidence? The claim itself has to be analysed and understood. Within the claim, identify one or more of the following:

- known scientific concepts
- variables
- measurable terms
- ideas related to concepts
- a term that is stated to influence another.

You should record all the information you collect during the investigation in a journal, including the process of developing a research question from a claim. This will be used in your research investigation report to address the ISMG characteristic about developing a research question from a claim. Write down all the elements found in the claim and try to categorise them using the above list. Each element within the claim may suit more than one category. Table 1.12.1 (page e96) outlines an example of analysing and categorising elements of a claim.

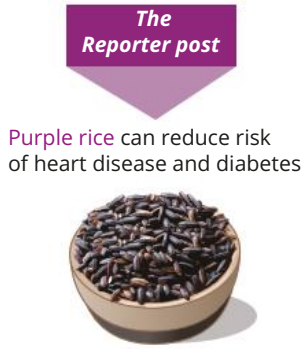


FIGURE 1.12.1 An article with a claim in the title.

TABLE 1.12.1 Examples of analysing and classifying elements of a claim

Example 1		
Claim	DNA, key to customising wellness, weight-loss solutions	**Classifying elements of the claim** DNA • known concepts – 3D structure includes proteins (e.g. histones), sequence of nucleotides, introns and exons • variable in numerous concepts (genes, protein synthesis, genetic regulation, inheritance, genetic identification technology e.g. electrophoresis) • measurable terms – genetic code, gene length • DNA is influenced by inheritance, also epigenetics Genetic screening • known concept • measurable • related to concepts (DNA fingerprinting, CRISPR, PCR, restriction enzymes)
Source and context of claim	An article from an online newspaper in reference to using genetic testing to screen for particular genes and customise lifestyle habits to improve health.	
Elements within the claim	• DNA • genetic screening (technology) • correlation between genes and weight loss • correlation between genes and health	

Example 2		
Claim	Adopting a healthy diet can help reverse risk of high triglycerides, despite genetic predisposition.	**Classifying elements of the claim** Genes • variable related to predisposition • concept related to gene regulation, predisposition, inheritance Alleles • known scientific concept in genetic disease, predisposition, mutations • measurable term (variation of allele, population frequency, percentage risk or risk factor) • influenced by mutations, inheritance Gene expression • measurable term (amount of product) • related to epigenetics, mutations, disease
Source and context of claim	An online article from an online, open-access medical information provider for professionals claims that a healthy diet can reverse the risk of high fat concentration in the blood of people with a genetic predisposition.	
Elements within the claim	• genes • alleles • epigenetics • gene expression	

If you unpack the claim into elements such as related terms and concepts, variables and measurable items, you can formulate questions using the elements within the claim. It is important to unpack and extrapolate elements that are scientific, meaning variables that are measurable, so that a valid scientific research question can be formed.

FORMING A QUESTION

A research question needs to be formed from the claim, because the variables stated in the claim may not be measurable or directly observable, and therefore they may not be scientific.

After extrapolating and unpacking the claim further into possible scientific elements, you can use each of these elements to form a research question (Table 1.12.2). It is best to formulate a number of questions related to the claim and write them all down in your journal. Each question must specify a dependent variable and enable a response that will evaluate the validity or the reliability of the claim.

This necessitates forming questions and using the elements of the claim in the phrasing of the research question. To complete this task, you will need to understand both the dependent and independent variables related to the claim, and the relationship between them. The claim must identify the dependent variable. The claim may also specify the independent variable. If the independent variable is not in the claim, it will be elsewhere in the material.

Some guidelines to help form a question are:

- search for the dependent variable in the claim, or refine it by rephrasing it into something measurable
- search for an element of the claim to be an independent variable and form a question around this, or identify it in the material
- phrase the question to ask if the independent variable will influence, cause or correlate with the dependent variable
- write a few different questions. Usually your questions improve as you write more.

Read through Table 1.12.2 to see examples of questions formed from the elements of the claims from Table 1.12.1.

TABLE 1.12.2 Examples of formulated questions from a claim

Example 1	
Claim	DNA is the key to customising wellness and weight-loss solutions.
Elements of a claim	**DNA** • DNA is influenced by inheritance, also epigenetics
Formulated questions	**A** Can genetic coding in the DNA affect individual weight-loss solutions? **B** Can genetic coding in the DNA affect weight loss? **C** Can genetic coding in the DNA affect physical health?

Example 2	
Claim	Adopting a healthy diet can help reverse the risk of high triglycerides, despite genetic predisposition.
Elements of a claim	**Alleles** • measurable term (variation of allele, population frequency, percentage risk or risk factor)
Formulated questions	**A** Can a healthy diet affect the genes of predisposed individuals affecting the risk of high triglycerides? **B** Is there a difference in high-triglyceride risk between individuals on healthy diets?

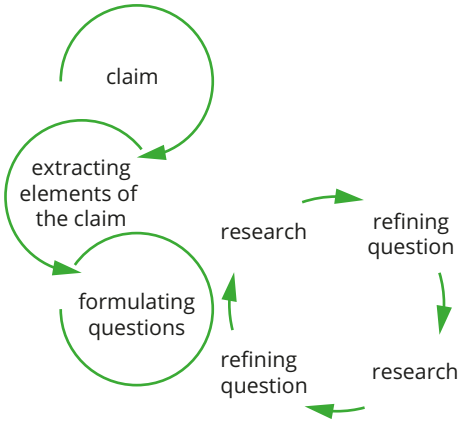


FIGURE 1.12.2 A chart of a common process for developing a research question. This cycle can be repeated as many times as necessary until a scientist is satisfied with the investigation question.

REFINING THE RESEARCH QUESTION

The process to developing a research question is often cyclical (Figure 1.12.2), and often the original question (or one of them) will be redeveloped or refined. As you research the background information related to your claim and proposed research questions, you will develop a greater understanding and may find it more appropriate to refine the question to be more specific.

The research investigation ISMG requires a conceptual explanation showing how the claim was developed into a research question, so record your background research, your development of understanding about the variables, concepts and relationships. Ensure you can justify a reason for the proposed relationship in the research question theoretically through scientific concepts. Following the step-by-step guide in Figure 1.12.2 will help.

The research question needs to be developed to the point at which exact data or evidence can be found regarding the variables in the question. Hence, it will develop into a research question when evidence from research can answer the question. Table 1.12.3 shows development of formulated research questions that were refined.

> The research question and the development of the research question from the claim using research, scientific concepts, knowledge and understanding is related to characteristics:
> - a carefully and deliberately constructed rationale identifying an easy-to-understand development of the claim from the research question
> - a research question that is clear, applicable and pertinent to the methodology
> - selection of adequate, applicable and pertinent resources.

TABLE 1.12.3 Development of the original formulated questions into research questions

Example 1			
Claim	DNA is the key to customising wellness and weight-loss solutions.		
Formulated questions	**A** Can genes affect individual weight-loss solutions?	**B** Can genetic coding in the DNA affect weight loss?	**C** Can genetic coding in the DNA affect physical health?
Refined research question	**A** Can variations of genetic code cause variations in metabolism?	**B** Can variations of genetic code cause variations in the metabolism of adipose tissue?	**C** Is there a correlation between gene variants (alleles) and cardiac health?

Example 2		
Claim	Adopting a healthy diet can help reverse the risk of high triglycerides, despite genetic predisposition.	
Formulated questions	**A** Can a healthy diet affect the genes of predisposed individuals affecting the risk of high triglycerides?	**B** Is there a difference in high-triglyceride risk between individuals on healthy diets?
Refined research question	**A** Can a healthy diet affect gene regulation in predisposed individuals and lower the risk of high triglycerides?	**B** Is there a significant difference in high-triglyceride risk between predisposed and non-predisposed individuals on healthy diets?

To write a specific and relevant research question as required by the ISMG characteristics, we need to address the definitions in the QCAA Biology syllabus of the terms 'specific' and 'relevant'.

- Specific means 'clearly defined or identified; precise and clear in making statements or issuing instructions; explicit'.
- Relevant means 'bearing upon or connected with the matter in hand; to purpose; applicable and pertinent; having a direct bearing on'.

Therefore, a specific research question must explicitly identify the dependent and independent variables and a relevant research question must be connected to the considered rationale and the topic of study.

1.12 Review

SUMMARY

- A claim is an assertion made without any accompanying evidence to support it.
- Research questions can be developed from claims by identifying the underlying scientific concepts and variables of a claim.

KEY QUESTIONS

Retrieval

1 Define claim.

2 State what a research question should explicitly identify.

Comprehension

3 Describe why a research question is refined.

4 Explain how refining a research question is related to finding evidence.

Analysis

5 Construct a question for the following claims.

- **a** Red wine stops cancer.
- **b** Chocolate kills dogs.
- **c** Global warming will kill domestic cats.

6 Specify how research and understanding will enable the research question to be relevant to the ISMG.

1.13 Finding and choosing suitable resources

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- distinguish between primary and secondary sources
- locate a range of primary and secondary sources
- determine the validity and reliability of a source.

When you gather scientific evidence for the research investigation, source it from reputable publications including:

- scientific journals, research papers and scientific reviews
- scientific articles written by organisations who apply scientific research to their industry
- commercial articles such as science magazines, newspapers and websites.

Your research investigation report must include a reference list of cited resources. The resources used should be sufficient and relevant. The QCAA Biology syllabus defines sufficient as 'enough or adequate for the purpose', and relevant as 'bearing upon or connected with the matter in hand; to the purpose; pertinent; applicable and pertinent; has direct bearing on'. Figure 1.13.1 points out the features of sources deemed 'sufficient' and 'relevant'.

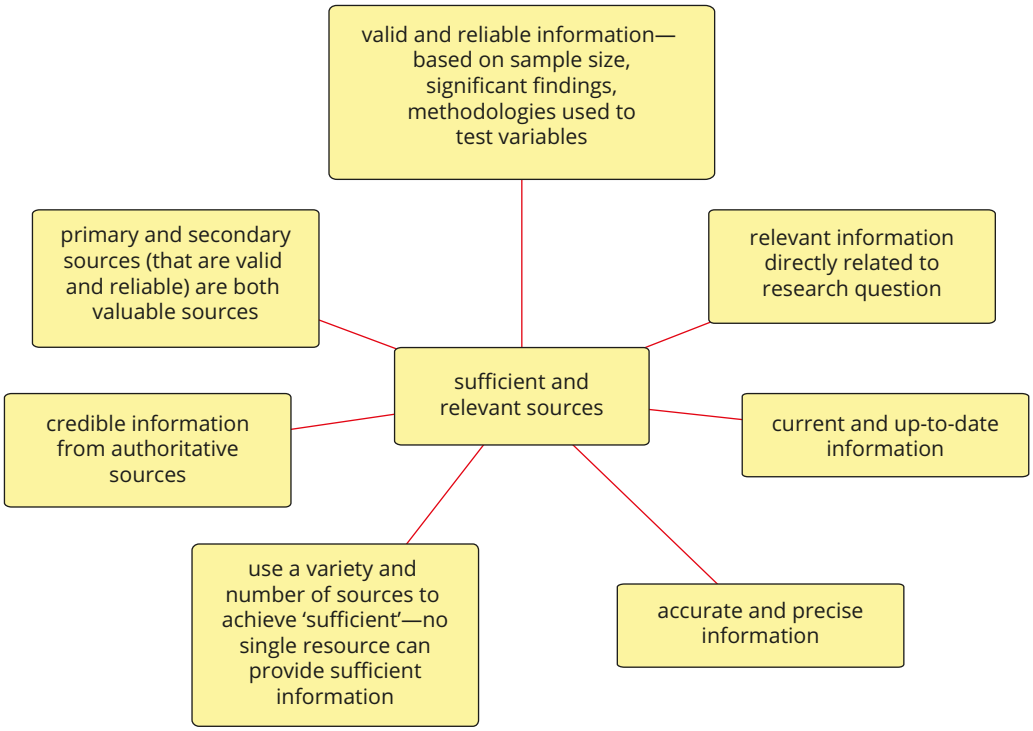


FIGURE 1.13.1 Features of sources suitable for the research investigation.

SOURCING INFORMATION

Consider whether the information you use is from a primary or secondary source.

Primary and secondary sources

Table 1.13.1 summarises the characteristics of primary and secondary sources. A primary source is the information from the original founder (or investigator) that discovered or measured the information. A secondary source is from anyone who uses information from others. For example, a scientist's journal article on the alleles associated with breast cancer is a **primary source**, while a general magazine article about breast cancer, written by a journalist and referring to the scientific study, is a **secondary source**.

TABLE 1.13.1 Summary of primary and secondary sources

	Primary sources	Secondary sources
characteristics	• first-hand records of events or experiences • written at the time the event happened • original documents	• interpretations of primary sources • written by people who did not see or experience the event • use information from original documents but rework it
examples	• results of experiments • scientific journal/magazine articles • reports of scientific discoveries • photographs, specimens, maps and artefacts • interviews with experts • websites (if they meet the criteria above)	• textbooks • biographies • newspaper articles • magazine articles • radio and television documentaries • websites that interpret the scientific work of others • podcasts

A primary source is written by the observer or witness of an event or the scientist who conducted the research. The information has only been processed by the original observer, so it is the least biased of all available sources of information. However, even primary sources may be biased, as the observer or researcher had to make choices related to the observation, control of variables, use of instruments and choices of processing.

Secondary sources of information are not eyewitness accounts but interpretations of events by other people. As second-hand information, their accuracy and reliability may be reduced, and events may be interpreted through the author's perception and bias. You should aim to use a wide range of data sources when using **secondary data**, to cross-check for accuracy, reliability and validity of information.

When searching for information and evidence, follow these guidelines.

1 Determine if it is a primary or secondary resource.
2 Confirm it is valid.
   a Check that it contains information that is specifically related to the claim.
   b Check that the evidence and information is pertinent to the variables in the research question.
3 Assess its reliability.
   a Is it current/recent information?
   b Is it up to date in its understanding of relationships?
   c Is the evidence equivalent to other sources?
   d Is it credible? Consider who the author is, and their qualifications and expertise.
   e How rigorous was the methodology? Consider what variables were controlled or measured.

Articles in scientific journals

Peer-reviewed (critically reviewed by other specialist scientists) scientific journals are excellent sources of information. Journals are a collection of scientific reports written by scientists to report the results of the research they conducted (Figure 1.13.2). The reports and articles found in scientific journals are primary sources. They follow a strict structure: an appropriate title; the names of the authors; an abstract; and the report, which includes an introduction, method, results, analysis, conclusion and reference list.

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Squeezing the Muscle: Compression Clothing and Muscle Metabolism during Recovery from High Intensity Exercise

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Abstract

The purpose of this experiment was to investigate skeletal muscle blood flow and glucose uptake in m. biceps (BF) and m. quadriceps femoris (QF) 1) during recovery from high intensity cycle exercise, and 2) while wearing a compression short applying ~37 mmHg to the thigh muscles. Blood flow and glucose uptake were measured in the compressed and non-compressed leg of 6 healthy men by using positron emission tomography. At baseline blood flow in QF ($P = 0.79$) and BF ($P = 0.90$) did not differ between the compressed and the non-compressed leg. During recovery muscle blood flow was higher compared to baseline in both compressed ($P<0.01$) and non-compressed QF ($P<0.001$) but not in compressed ($P = 0.41$) and non-compressed BF ($P = 0.05$; effect size = 2.74). During recovery blood flow was lower in compressed QF ($P<0.01$) but not in BF ($P = 0.26$) compared to the non-compressed muscles. During baseline and recovery no differences in blood flow were detected between the superficial and deep parts of QF in both, compressed (baseline $P = 0.79$; recovery $P = 0.68$) and non-compressed leg (baseline $P = 0.64$; recovery $P = 0.06$). During recovery glucose uptake was higher in QF compared to BF in both conditions ($P<0.01$) with no difference between the compressed and non-compressed thigh. Glucose uptake was higher in the deep compared to the superficial parts of QF (compression leg $P = 0.02$). These results demonstrate that wearing compression shorts with ~37 mmHg of external pressure reduces blood flow both in the deep and superficial regions of muscle tissue during recovery from high intensity exercise but does not affect glucose uptake in BF and QF.

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Introduction

Skeletal muscle blood flow incorporates a key role in aerobic muscle metabolism matching the delivery of oxygen and energy substrates for energetic demands, as well as the transportation of waste products and heat from the muscle tissue. The response of skeletal muscle blood flow and metabolism at the onset of and during exercise are well documented. In general, muscle blood flow increases rapidly with an exercise-depended plateau after approximately 30 s of exercise [1,2]. Muscle blood flow is heterogeneously distributed [3] in a manner that it is higher in the deeper compared to the superficial parts of the m. quadriceps femoris [4].

Another point is that blood glucose concentration plays an important role in restoring muscle glycogen during recovery from exercise [5]. Unfortunately, little is known about the crucial role of blood flow and its association to glucose uptake, during recovery from high intensity exercise [6] and so far no study investigated this matter in connection with the application of compression clothing.

In the past two decades, various forms of compression clothing have been applied by elite and recreational athletes due to accumulating evidence regarding the possible performance [7,8] and recovery [9–11] enhancing properties. Early research showed that the application of 15 mmHg external pressure while lying in supine position reduces the cross-sectional area of the venous systems (from 2.65 cm^2 to 0.53 cm^2) thereby elevating mean linear blood flow velocity (from 0.5 cm/s to 2.5 cm/s) [12] and thus reducing contact time and thrombogenesis [13]. The application of compression seems to enhance muscle blood flow [14] and has been associated with enhanced clearance of metabolites, such as blood lactate [15,16]. However, it also has been suggested that reduced levels of blood lactate are attributable to a greater retention of the molecule within the muscle [15,16]. In this context two recent literature reviews summarized the effects of compression on metabolite clearance to be controversial [17,18]. In this regard, especially clothing with a high level of compression (>30 mmHg) has not been investigated regarding its effects on blood flow and glucose uptake during recovery.

FIGURE 1.13.2 An extract of an article in a scientific journal of a research report written by scientists.

You may find it difficult to access scientific journals, as many journals require subscriptions or financial membership. However, some journals are free. Figure 1.13.3 (page e102) provides ideas for accessing scientific journals.

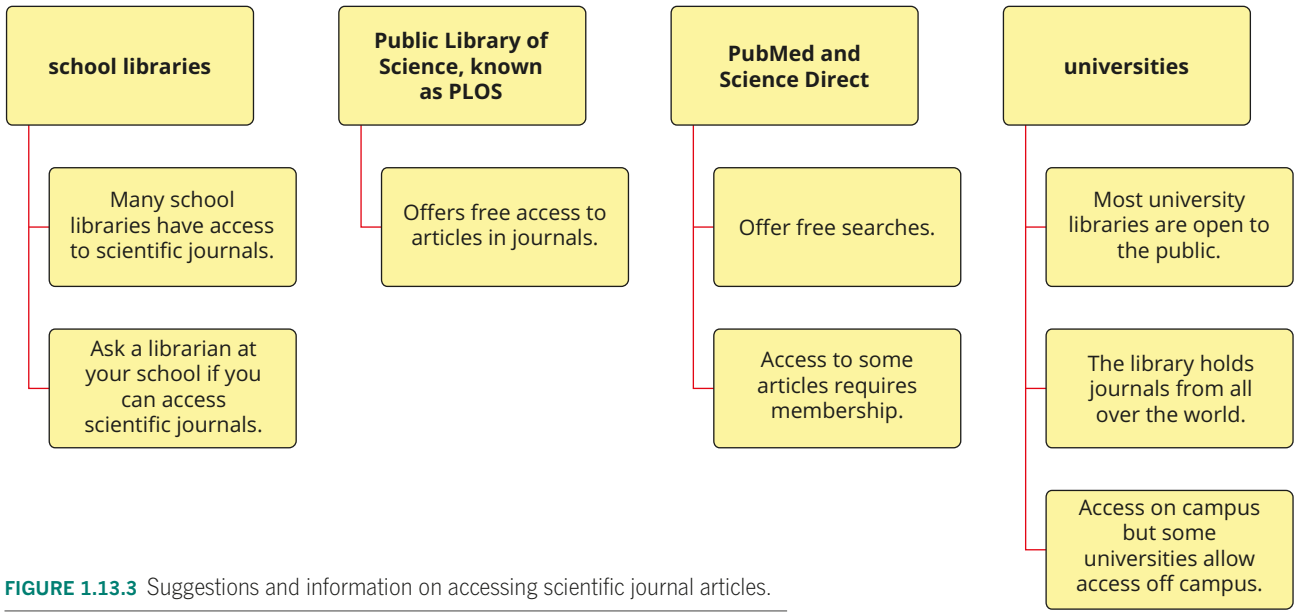


FIGURE 1.13.3 Suggestions and information on accessing scientific journal articles.

Table 1.13.2 outlines some advantages and disadvantages of sourcing and using articles in scientific journals for the research investigation.

TABLE 1.13.2 Advantages and disadvantages of using scientific articles for the research investigation

Advantages	Disadvantages
• written by experts • authoritative information (peer-reviewed) • most current information • logical, organised layout • content is relevant to the topic • contain an abstract that summarises all information in the article (if you don't find the information in the abstract, the article is not relevant) • primary source	• information is complex and challenging to understand—complex language and advanced processing and analysis of data • requires an understanding of scientific language and numeracy to understand • may be time-consuming to read and analyse • well-established concepts do not have recent published articles • may be difficult to locate

Books and physical publications

Secondary sources such as good science magazines and books are valuable sources of secondary information.

The first source you should use is your textbook. The language and concepts are presented specifically for high school students. In addition, the textbook addresses the syllabus objectives. Non-fiction books and magazines will probably be commonly used resources for the research investigation. Common commercial science magazines in your school library could include *New Scientist*, *Cosmos*, *Scientific American* and *The Helix* (Figure 1.13.4). Table 1.13.3 outlines some advantages and disadvantages of non-fiction books as sources for your research.

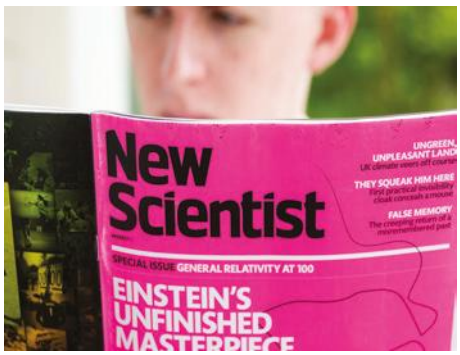


FIGURE 1.13.4 A science magazine you might find in your school library.

TABLE 1.13.3 Advantages and disadvantages of using book resources for the research investigation

Advantages	Disadvantages
• may be written by experts • potentially authoritative information • logical, organised layout • content is relevant to the topic • contain table of contents and index to help find relevant information • easily located in libraries • written in language that is understandable	• may not have been published recently • can be accessed by only one person at a time • may have more bias than primary sources • may be less reliable than primary sources

Searching online

Online sources should include online scientific organisations such as CSIRO, NCBI, AIMS, Bioscience (Oxford Academic) and other university publications and presses. Many government and privately funded science organisations publish material, as do not-for-profit scientific organisations. Websites may direct you to magazines and scientific journals, such as those described above, the news, podcasts, blogs and videos (institutional, company and personal).

Information on the internet must be very carefully scrutinised. The openness and ease of publishing on the internet means information may not be valid and reliable. Use the earlier guidelines above to help you evaluate sources. Table 1.13.4 outlines advantages and disadvantages of locating and using information online.

When searching for relevant information, you will need to use a search engine and use appropriate search terms. Here are some tips for searching online.

- Break your search statement into concepts and key words.
- Find synonyms, other related terms and concepts that apply to the topic.
- Create concepts of 1–3 words to enter into the search engine.
- Try different combinations of terms.
- Don't settle for the first sites on the list or your first attempt. Look beyond the first page of results.
- Look through the results for sites from science organisations and research institutions (e.g. CSIRO, WEHI, NIH; .gov, .org), universities (.edu) and science journals and magazines.

TABLE 1.13.4 Advantages and disadvantages of using internet resources for the research investigation

Advantages	Disadvantages
• quick and easy to access • allows access to hard-to-find information • allows access to the whole world; millions of websites • information is potentially more up to date • may be interactive and use animations to enhance understanding	• can easily become distracted by non-relevant information • a lot of 'junk' sites and potentially more biased material • need to discern search engine results to find most useful sites • cannot always tell how up to date information is • can be hard to tell who has responsibility for authorship • information may not be well ordered • may not be reliable, valid or credible

Overview of resources

Your textbook should be your first source of reliable information. Other information should be consistent with this. Articles published in journals and magazines often present findings of new research, which may or may not be confirmed later, so be careful not to treat such sources of information as established fact. Scientific journals are peer-reviewed, which make them more credible than other sources.

SKILLBUILDER

Evaluating sources for validity and reliability

Determining the validity and reliability of a source can be a challenging task. For some sources, it is easy to find details about the author, evidence and currency, while others only contain content and do not offer any other details. Another difficulty is that, when learning about a new topic or concept, we are all novice learners so it can be challenging to tell if a source is valid or not.

These tables outline step-by-step examples of evaluating a resource for a claim about the relationship between single nucleotide mutations (known as single nucleotide polymorphisms—SNPs) in genes and disease.

SOURCE EVALUATED: 'Non-Synonymous and Synonymous Coding SNPs Show Similar Likelihood and Effect Size of Human Disease Association'—scientific research article (note: 'non-synonymous' refers to non-silent mutations; 'synonymous' refers to silent mutations).

Criteria		Decision	Support/justification
Primary or secondary	Is this an eyewitness account or a second-hand source?	Secondary	• research review article published in PLoS • primary data from 2113 research articles were analysed
Validity	Does it contain information that is specifically related to the claim?	Yes	• outlines information directly related to types of SNPs (non-synonymous and synonymous) and association to disease
	Is the evidence and information pertinent to the variables in the research question?	Yes	• information, experiment and research analysed, statistical analyses are specific to types of SNPs (IV) and association to disease (DV)
Reliability	Is it current/recent information	Yes	• published October 2010
	Is it up to date in its understanding of relationships?	Yes	• information outlines the developing understanding of genetic mutations, and how silent (synonymous) and non-silent (non-synonymous) mutations are equivalently associated with disease
	Is the evidence equivalent to other sources?	Yes (for recent articles)	• results are relatively new; however, when searching and scanning other research article abstracts (post-2010), equivalent conclusions have been published
	Is the author(s) credible?	Unknown	• Authors are Chen Rong, V. Eugene Davydov, Marina Sirota and Atul Butteare—expertise unknown • This work was supported by Lucile Packard Foundation for Children's Health, National Institute of General Medical Sciences (R01 GM079719), National Library of Medicine (R01 LM009719), National Cancer Institute (R01 CA138256), Howard Hughes Medical Institute, Pharmaceutical Research and Manufacturers of America Foundation, and Hewlett Packard Foundation
	Is the sample size known?	Yes	• 21 429 SNPs were analysed across 2113 published research articles
	What variables were controlled or measured?	Unknown	• numerous beyond possibility to list them all • the variables would not have been controlled or measured equivalently across all articles • numerous variables across many articles that I would not understand

A judgement could be made about this source such as: 'The information and evidence was published in a peer-reviewed journal article that is concurrent and with variables of experimentation that can be determined and directly related to the claim and research question. The results are relatively new and are substantiated by an exceptionally large sample size and other articles.'

SOURCE EVALUATED: Bioninja degeneracy—web page

Criteria		Decision	Support/justification
Primary or secondary	Is this an eyewitness account or a second-hand source?	Secondary	• general info with schematic diagrams
Validity	Does it contain information that is specifically related to the claim?	Yes	• description of silent mutations
	Is the evidence and information pertinent to the variables in the research question?	No	• information refers to protein structure rather than disease
Reliability	Is it current/recent information?	Unknown	• no dates provided; however, corroborates with other sources
	Is it up to date in its understanding of relationships?	No	
	Is the evidence equivalent to other sources?	Yes	• polypeptide sequence is unchanged
	Is the author credible?	Unknown	• no author published
	Is the sample size known?	Unknown	• not published
	What variables were controlled or measured?	Unknown	• not published

The judgement of this second source could be as follows: 'No evidence is provided about the source of information, author (their expertise) or dates of claims. The information is partially concurrent and directly related to the claim and research question. The source is somewhat valid but may not be reliable.'

Test the skill

In researching about the claim 'Are silent mutations truly silent', the article *Silent mutations speak up* was found at http://www.nature.com/news/2006/061221/full/news061218-12.html. Evaluate this source in relation to its usefulness in investigating the claim. Use a table like those in the examples above to make a judgement of the validity and reliability of the source.

SOURCE EVALUATED: 'Silent mutations speak up', http://www.nature.com/news/2006/061221/full/news061218-12.html

Criteria		Decision	Support/justification
Primary or secondary	Is this an eyewitness account or a second-hand source?		
Validity	Does it contain information that is specifically related to the claim?		
	Is the evidence and information pertinent to the variables in the research question?		
Reliability	Is it current/recent information?		
	Is it up to date in its understanding of relationships?		
	Is the evidence equivalent to other sources?		
	Is the author credible?		
	Is the sample size known?		
	What variables were controlled or measured?		

1.13 Review

SUMMARY

- Scientific evidence for the research investigation can be sourced from numerous publications including scientific journals, scientific articles and commercial articles.
- A primary source is written by an observer or eyewitness of an event, or the scientist who conducted the research.
- A secondary source is a document that refers to or analyses a primary source.

KEY QUESTIONS

Retrieval

1 Decide whether each of the following is a primary or a secondary source.

a a newspaper article about global warming

b an experiment to investigate chemical changes when mixing combinations of chemicals

c an interview with a forensic scientist about using science in tracking criminals

d a website with information about genetic engineering

Comprehension

2 Use a graphic organiser to depict the characteristics of primary and secondary sources, and when to use each.

Analysis

3 You are learning about genetically inherited diseases and are searching for facts about cystic fibrosis. Deduce which of the following resources should be the most valid, after applying the evaluation step-by-step guide in the Skillbuilder on page e104.

A the book *Cystic Fibrosis*, published in 1997

B the article 'Living with cystic fibrosis' published in the *Daily Mail* on 23 February 2008

C the website www.cysticfibrosis.org.au, accessed on 30 October 2015

1.14 Taking and organising notes

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- use your scientific journal to take and organise notes, and refine experimental procedures
- paraphrase information found in primary and secondary sources.

Once all the research, synthesising of information and work has been completed in your journal, there will be no need to produce new work. It has already been completed, so you can write your final research investigation report. At this point, your journal should be full of information, but it may be scattered across numerous pages and it may be challenging to find some crucial information. Your note-taking in your journal needs to be organised.

Scientists organise their journal notes to record the:

- date of journal entries
- journal entries for:
  - ideas, observations, proposals and questions
  - research background information for ideas, observations, proposals and questions
  - refinements
  - personal explanations of information, concepts, ideas, observations, proposals and questions (this often includes diagrams)
  - results, data and evidence
- origin of information (recording sources).

RECORDING DATE OF JOURNAL ENTRIES

Always place the date at the beginning of the work you are doing each day. This is a professional practice used to catalogue and file information. When you are trying to find previous completed work, it is easiest to find it by date or topic. This is a simple yet effective filing system.

RECORDING JOURNAL ENTRIES

Each journal entry should follow a cataloguing system; most typically this is the date and a title. A cataloguing system enables quick reference and helps you to find information when you are searching for it later. As you take notes from different sources, always record the source of information, for example, the title, URL, author, page.

Ideas, observations, proposals and questions

You may be surprised how often simple ideas, observations, proposals and questions will influence, direct and help your research days or weeks later.

Your ideas, observations, proposals and questions could be related to:

- the variables or concepts involved in your investigation
- new terms you were exposed to and do not understand
- data or evidence you do not currently understand but that are important to some part of the investigation
- statistics about significance in the analysis of some information
- personal ideas and proposals about possible future research and questions.

Record all of these in your journal. Later they may:

- save you time by:
  - preventing you from researching the same idea twice
  - helping you to link ideas from one day or week to the next when you return to your work
  - suggesting guidance for research and queries that are related

Many professionals use a couple of cataloguing systems when they are recording information in their journals. They usually record the date of entries and provide a title for their entries. The titles are often related to their own work and objectives (categories) rather than the title of the source. The title of the source is recorded when the origin is recorded. The most common cataloguing techniques are dates, titles and recording the source.

- provide links between concepts in the future that are currently unknown
- provide answers (or partial answers) to future issues, conceptual blocks and questions
- develop your understanding of unrelated concepts that become relevant later.

The content and recordings in your journal will not be in a logical conceptual order, the way that your class notes or a textbook are. This is because you are researching unfamiliar knowledge and you won't know how it all fits together until the research is complete. This is why it is important to catalogue your journal entries.

Researching background information

Information taken from a source should be rewritten or summarised in your own words in your journal. Avoid copying information verbatim, so you are not tempted to plagiarise when you write up the research investigation.

It can sometimes be difficult to rewrite information in your own words, especially if it is already well-expressed and concise. Before you write and record the research information, read the material and grasp its understanding. Without referring back to the source, write notes in your journal in your own words. Use multiple sources and a dictionary as references for information. In your journal, your notes should be detailed with extended explanations that you will fully understand when you refer back to them at a future date. See Worked example 1.14.1 for a few examples of rewriting information.

Worked example 1.14.1

REWRITING INFORMATION FROM SOURCES

An information source states: 'It can be expected that the days will be warm and the nights will be cool over the next three days due to the high-pressure system moving across the region.' Rewrite this information in your own words.	
Thinking	**Working**
Can swap the order of information, the object (the warm days and cool nights) with the reason (high-pressure system).	Due to the high-pressure system moving across the region, the days will be warm while the nights will be cool.
Can change numerous words, such as adjectives, verbs, adverbs and nouns.	It has been predicted that during the daytime it will be warm while the evenings will be cool for about three days, as a high-pressure system travels over the region.
Can elaborate on the information to explicitly outline explanations.	The weather forecast expects (made a prediction based on computer simulations) that the temperature during the day will be warm (22–25°C) and the evenings will be cool (below 12°C) due to a high-pressure system (weak high-pressure system), which dissipates clouds and allows direct sunshine to warm the Earth's surface. However, in the evenings this system brings cool air from higher altitudes that are no longer warmed by the sun.

➤ Try yourself 1.14.1

REWRITING INFORMATION FROM SOURCES

An information source states: 'This will be followed by a low-pressure system which will bring cooler days and not-so-cool nights.' Rewrite this information in your own words.

Personal explanations and interpretation

The journal is a valuable tool that is personal to you. Use a variety of written notes, drawings and schematics (e.g. flow charts) that you have personalised to help you understand the concepts and relationships.

Depending on the information or concept, one form of recording information can provide more details than another. Also, recording the details is more than just recording the facts. For example, it can be the order of events, their location or their physical orientation. Use a variety of ways to record or present the information to capture more comprehensive details. Figure 1.14.1 presents the same information in a few different ways.

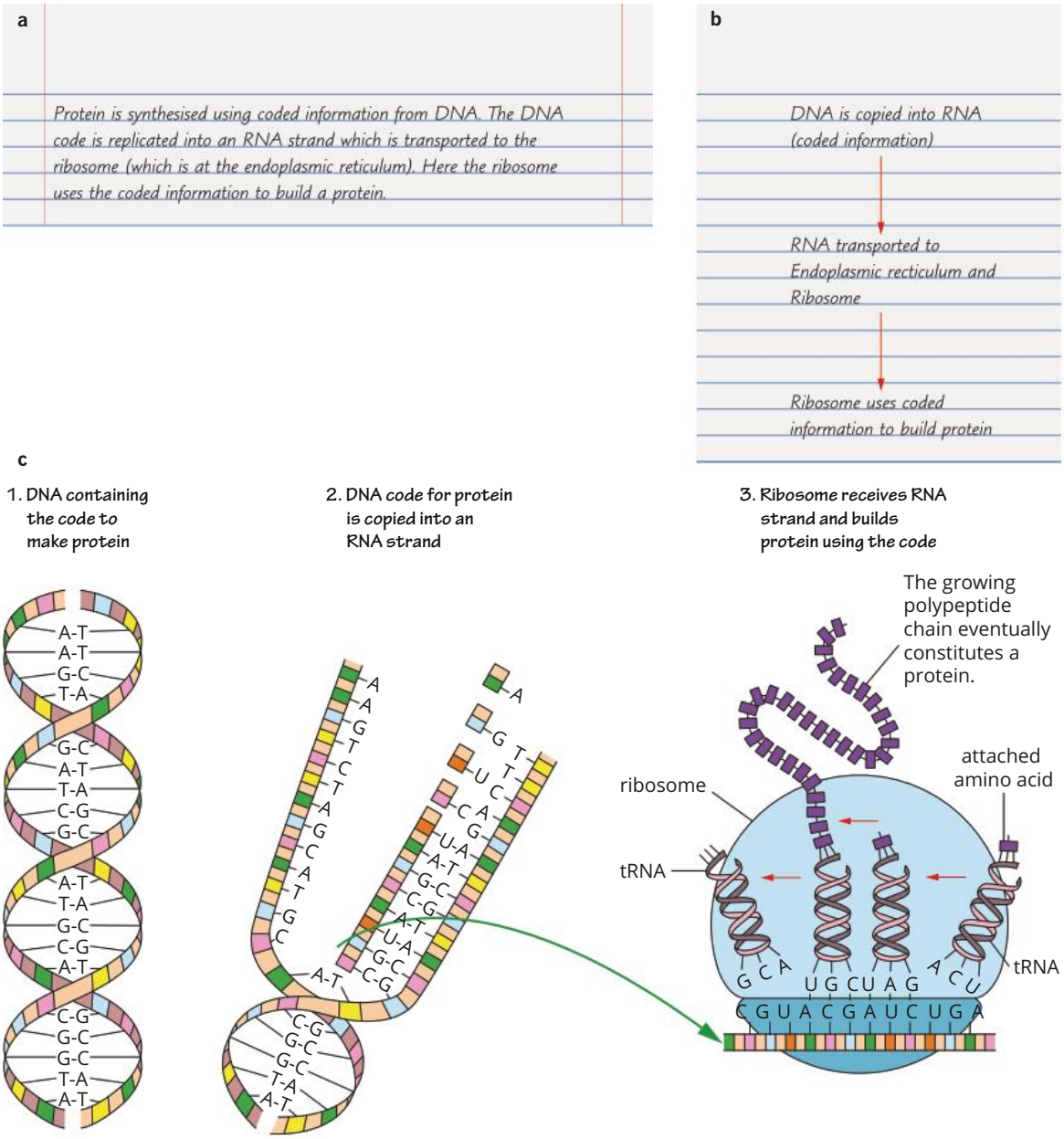


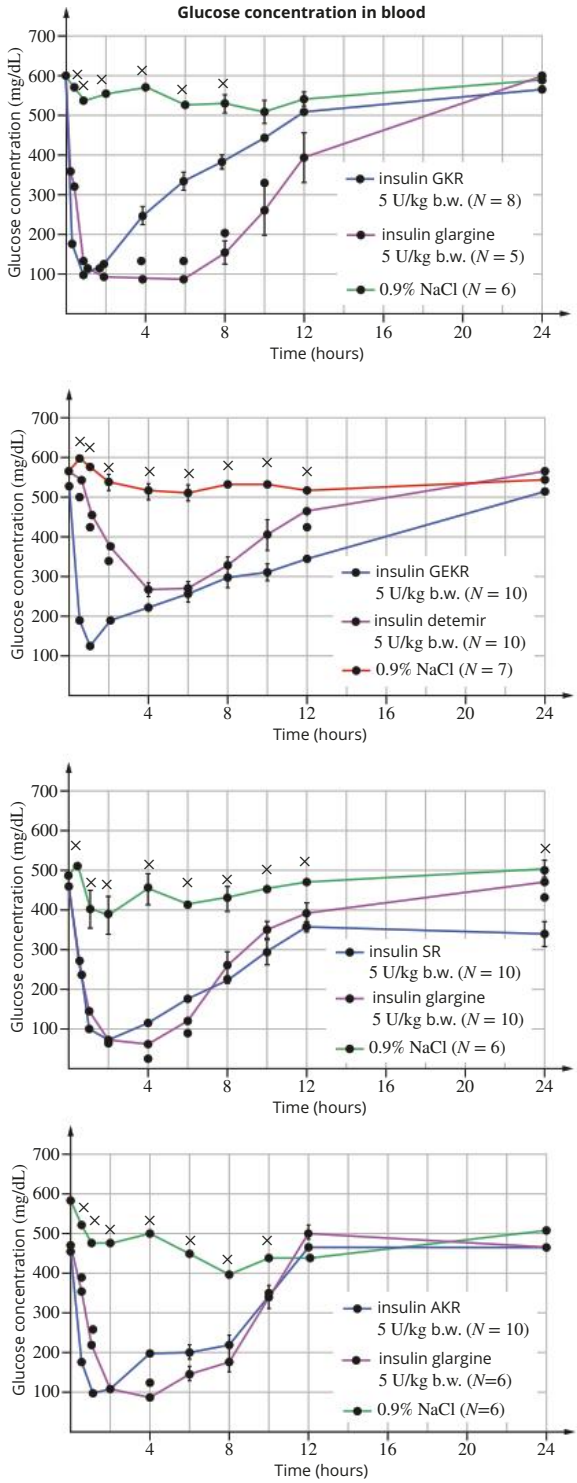
FIGURE 1.14.1 Three ways of recording the same information in a journal: (a) written summary on protein synthesis; (b) protein synthesis in a flow chart as a schematic; (c) diagram explaining protein synthesis.

Results, data and evidence

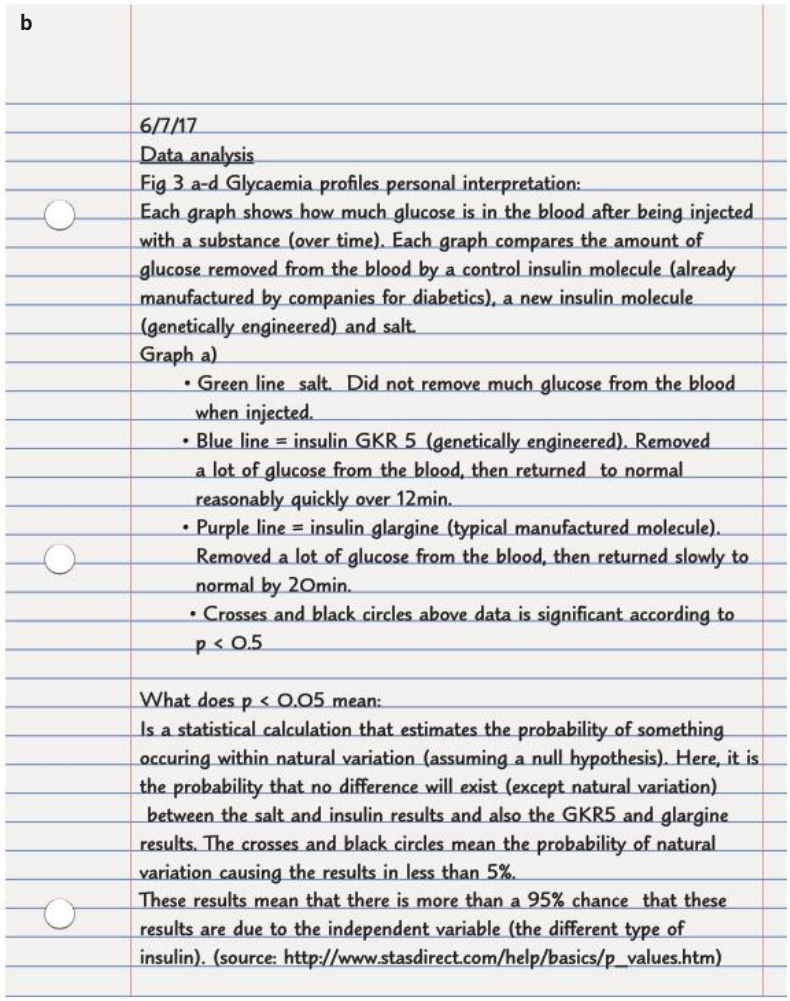
When you come across scientific results or data in a resource, it is important to record the values accurately or reproduce them in the journal. Also, interpret them and summarise them in your own words. Figure 1.14.2 illustrates an example of a journal entry written under an accurate redrawing of scientific results. A summary of scientific results or data should include:

- the trend or pattern
- the comparison or difference between one set of values and another
- the statistics used to establish significance and also the author's interpretation
- specific details and your own interpretation of significant values.

a



b



6/7/17

Data analysis

Fig 3 a-d Glycaemia profiles personal interpretation:

Each graph shows how much glucose is in the blood after being injected with a substance (over time). Each graph compares the amount of glucose removed from the blood by a control insulin molecule (already manufactured by companies for diabetics), a new insulin molecule (genetically engineered) and salt.

Graph a)

- Green line salt. Did not remove much glucose from the blood when injected.
- Blue line = insulin GKR 5 (genetically engineered). Removed a lot of glucose from the blood, then returned to normal reasonably quickly over 12min.
- Purple line = insulin glargine (typical manufactured molecule). Removed a lot of glucose from the blood, then returned slowly to normal by 20min.
- Crosses and black circles above data is significant according to $p < 0.5$

What does $p < 0.05$ mean:

Is a statistical calculation that estimates the probability of something occuring within natural variation (assuming a null hypothesis). Here, it is the probability that no difference will exist (except natural variation) between the salt and insulin results and also the GKR5 and glargine results. The crosses and black circles mean the probability of natural variation causing the results in less than 5%.

These results mean that there is more than a 95% chance that these results are due to the independent variable (the different type of insulin). (source: http://www.stasdirect.com/help/basics/p_values.htm)

FIGURE 1.14.2 The interpretation of original primary evidence from a research journal article: (a) primary evidence displayed in graphs with statistics; and (b) student's interpretation in their journal.

When making journal entries about scientific results, it is important that you can understand, analyse and interpret the statistical method used (e.g. *P* value, confidence interval, standard error). You will most likely have to conduct further research to understand the statistics, their meaning, and the author's interpretation of the results as you will come across new statistical calculations you haven't seen previously. Your research investigation report will have to explain the relevant data that you will use to form a conclusion. To choose the relevant data (or results) you will have to understand it, so only the pertinent data is chosen relevant to the relationship in the research question. Also, the report will have to be written succinctly in your own words. Understanding the data will help achieve this and avoid plagiarism.

Recording the origin of information

As you conduct your research, it is important to write down the source of the information. This will enable you to return to the source later to continue researching, collect further information or check details. You will also be required to produce a reference list in the report, and it will save time if you have already recorded the source of the information.

Table 1.14.1 shows what information to record about sources you use.

TABLE 1.14.1 Information that should be recorded about sources used in the research report

Books	Online
• author(s) • title • date of publication • publisher • place of publication • page(s)	• author(s) or name of organisation • title • date website was written or updated • date website was accessed • website address (URL)

1.14 Review

SUMMARY

- There is no single method for taking and organising notes.
- A scientific journal enables scientists to record and organise their notes.
- Scientists continually revise and refine their experiments on the basis of previous experimentation and results.

KEY QUESTIONS

Retrieval

1 List the information that should be recorded about a source in your journal.

Comprehension

2 Explain the benefit of keeping a scientific journal.

3 Explain two benefits of interpreting and summarising scientific results in your own words.

Analysis

4 Rewrite the following text in your own words.

a A group of patients was administered adrenaline during a cardiac arrest to determine if adrenaline would improve patient outcomes. There was no statistically significant improvement in patient outcome between patients who received adrenaline and those who did not.

b A team of scientists from the Oregon Health and Science University and Korea's Institute for Basic Science successfully repaired a defective gene in human embryos. The gene that was targeted caused a heart defect, known as hypertrophic cardiomyopathy, which causes sudden death in young adults.

c This study found that water temperature had no significant effect on the number of bacteria remaining on hands after washing. Rather, the length of time spent scrubbing hands, as well as the vigour of scrubbing, had the most effect on removing bacteria from hands.

1.15 Writing a report for the research investigation

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- write a report for your research investigation
- write a literature review for your research investigation.

There are many ways to present your report of your research investigation. A written report should be 1500–2000 words long and can take the format of a literature review, an empirical essay and an annotated bibliography. Alternatively, you can create a poster or a multimodal presentation of 9–11 minutes in length. This is not an exclusive list. Even though there are many ways to present your report, it must follow a scientific genre and meet the requirements of the QCAA Biology syllabus.

Writing a research investigation report requires scientific communication and you will need to follow scientific genre conventions. An explanation of these requirements has been provided in Module 1.11. A report should communicate information in a logical sequence (introduction, body and conclusion) and may contain subheadings.

Table 1.15.1 summarises the characteristics of the genres for presenting the research investigation. This module focuses on the literature review.

TABLE 1.15.1 Summary of features of presentation genres for the research investigation

Science presentation genre	Brief description	Features
literature review	• evaluates information related to the selected topic found in a publication • gives a theoretical base for the claim (question), and analyses and interprets information and data related to the claim • points out the strengths and weaknesses of the claim	• abstract • introduction to topic, providing context • discussion of information/data • analysis of information/data • evaluation of information/data • conclusion related back to claim • paragraphs that flow in a logical development of ideas • subheadings
empirical essay	• very similar to a literature review	
annotated bibliography	• notes, comments and explanations about article(s) • evaluates of a claim after investigating how other sources treat the claim	• citation • introduction to the citation • aims, research methods, scope • discussion of usefulness to your research topic • limitations • conclusions • reflection—explain how the citation contributes to your topic or fits in with your research
poster	• presents ideas concisely and clearly • ideas are elaborated on in the accompanying oral presentation	• includes all the above • visual, oral and text presentation • uses font size, colour, dot points, subheadings and logical flow of information to deliver information effectively

LITERATURE REVIEW

A literature review usually includes an introduction, a body and a conclusion. It critically analyses information and convinces the reader of the significance or importance of the topic being investigated. This is achieved by presenting information in a logical sequence that guides the reader through the material to understand its significance. The research question provides the foundational direction and guidance of the literature review.

Common elements and attributes

Not all elements of a full literature review will be appropriate for the research investigation. The literature review will be limited to the word count and ISMG characteristics in the syllabus. Choose to cover some of these elements of a literature review:

- determine the current understanding on the topic
- provide an overview of key concepts (relevant to the research question)
- identify important relationships between variables (specific to variables that influence the independent or dependent variables stated in the research question)
- identify strengths and weaknesses of evidence in the information used for the above points
- identify any gaps in the research
- identify any conflicting evidence.

Common attributes of literature reviews include:

- critical analyse of evidence—the meaning of the holistic evidence is established, including an account of the statistical processing of data used (includes an analysis, methodologies, samples, results, data processing and interpretations)
- discrimination between relevant and irrelevant sources and content—only relevant sources are used in literature reviews
- structural organisation—the structure of the review is logical (planned sections and sequence of information), informational and convincing (based on thorough analysis of evidence).

Critical analysis

One of the key qualities of a literature review is that it is a critical analysis of the evidence to communicate a true understanding of the 'big picture' about a topic. It does not just summarise information. Very few phenomena are witnessed in science, especially biological science, so facts are not exactly known. Science is more about models, theories, laws and principles and their continual development. The literature review should critically analyse evidence, the validity, reliability and limitations, as well as the gaps and conflicts to convince the reader about the current state of a large jigsaw puzzle of conceptual relationships.

Relevant sources

The ISMG for the research investigation outlines expectations for the discrimination of relevant sources—it must be sufficient. The term 'sufficient' is defined by the QCAA Biology syllabus as 'enough or adequate for the purpose'. No exact number of sources is required, as this can be different for each student. The purpose of the research investigation is to evaluate the claim. If the number of articles enables a justified conclusion to be drawn about the research question and explores both sides of the argument, then the number of sources is sufficient.

The critical analysis should be thorough, identifying patterns, trends and relationships that are related to the variables in the research question. It should clarify where sources agree and disagree, and provide a complete identification of limitations. The analysis of limitations will outline their influence on interpretations of results and also the conclusions (primary or secondary) of your investigation. It is important that when analysing data and its limitations, it is not done selectively to demonstrate what the author wants to show. Such bias will result in an inappropriate and erroneous conclusion that is invalid. Quality scientific analysis is open to any result.

Structural organisation

The structural organisation is evident by the sequential flow of justified scientific arguments and their support by sound reasons and evidence. Scientific arguments that are usually found in a literature review include:

- identifying or explaining whether or not a pattern, trend or relationship was observed between the independent and dependent variables in the research question
- if a relationship is found, a description of what kind of pattern exists and the conditions under which it exists
- acknowledgement and explanations of any **deviations** in the data or information
- identification of any limitations in the data or information
- if limitations are found, explanations of why and how the limitations affect the validity.

Your analysis of information may also include an evaluation of the methodology used by authors to obtain their data or information.

Conclusion and evaluation

The conclusion should include a discussion of the features of the evidence that limit its usefulness. For example, did the sampled population reflect the population referred to in the research question? Is the data from measured samples or from estimated models? Did the different studies occur under different conditions or categorise the independent variables differently so comparison was impossible?

These questions require a justified discussion that evaluates the reliability and validity of evidence. Therefore, it is important to discuss the limitations of each source. You can do this by:

- evaluating the method of evidence collection
- identifying issues that could affect validity, accuracy, precision and reliability of evidence
- stating sources of systematic and random errors
- recommending improvements to the evidence to improve validity.

In the conclusion, you should recommend improvements to the investigation that are linked to the evidence and would address the limitations and gaps in knowledge that have been identified during the research investigation. Your suggestions must be connected to the claim and allow further investigation.

Communication and structure of the report

This section provides a guide to writing an appropriate scientific report and some of the general conventions that need to be followed for a literature review. Once a plan for the literature review has been developed, personally set word limits assigned to each section. Figure 1.15.1 will provide a guide. The notes in your research journal will give you a good idea of which sections will require more words for explanations than others. The journal will also help you make decisions on which figures, data and evidence to include and also how many words may be required to elaborate on the evidence. Distribute the total word count across all sections you plan to include in the literature review, assigning a word limit for each section.

The personally set limits are not binding. As you complete your review, you can alter the word guide and distribution of the word count if you feel it is necessary. However, make sure the word limit outlined in the syllabus is never exceeded.

Planning the research investigation report will help address the syllabus ISMG. Figure 1.15.1 illustrates how planning can to be done to ensure the syllabus ISMG characteristics are addressed.

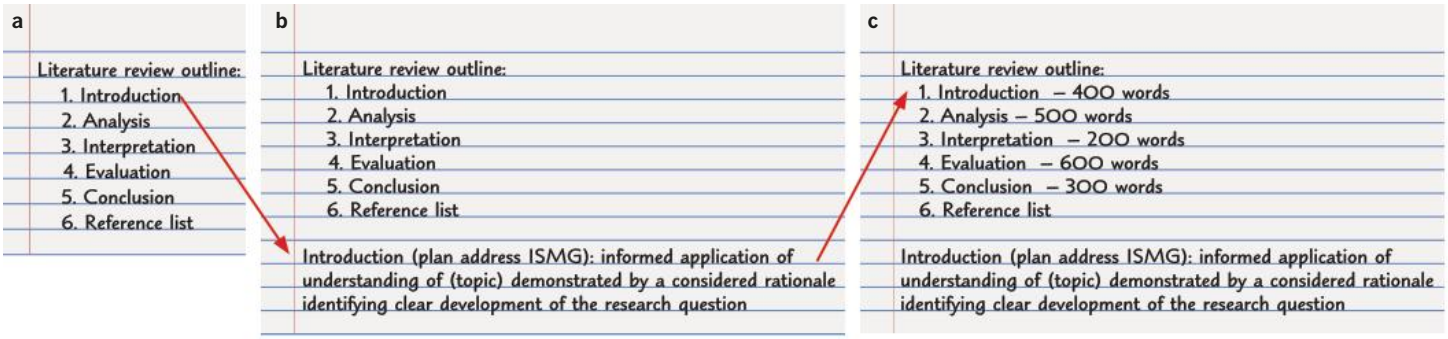


FIGURE 1.15.1 Planning the literature review. (a) A planned outline of the research investigation report to ensure the syllabus ISMG characteristics are addressed. (b) Assigning the ISMG characteristics to the sections in the planned outline. (c) The planned word count guide.

Editing the report

Edit the report after you have completed it. Editing is an important part of the process. When editing your report, save new drafts with a different file name and always back up your files in another location.

Pretend you are reading your report for the first time when editing. When reading your own work, do not read what you intended to write, but what you have actually written. Reading the report out loud to yourself will help you read it more critically.

When editing, look for content that is:

- ambiguous or unclear
- repetitive
- awkwardly phrased
- too lengthy
- not relevant to your research question
- poorly structured
- lacking evidence
- lacking a reference (if it is another researcher's work).

1.15 Review

SUMMARY

- There are several genres that can be used to report a research investigation, such as a literature review, empirical essay, poster presentation and annotated bibliography.
- A literature review critically analyses information and convinces the reader of the significance or importance of the topic being investigated.

KEY QUESTIONS

Retrieval

1 Describe the conventional flow of information through a literature review.

Comprehension

2 Explain the purpose of a literature review.

3 Summarise how you would achieve an analysis of the methodologies, samples and results in a literature review.

PART D EXAMINATION

The examination is set externally by the QCAA and covers both Unit 3 and Unit 4 of the Biology syllabus. The examination accounts for 50% of the total mark. The examination is delivered by two papers.

The QCAA defines an examination as a supervised test that assesses the application of a range of cognitions to one or more provided items such as questions, scenarios and/or problems. Students complete their responses individually, under supervision and in a set time.

The assessment objectives are to:

1 describe and explain
2 apply understanding
3 analyse evidence and identify trends, patterns, relationships, limitations or uncertainty
4 interpret evidence, analyse and draw conclusions.

The table below outlines key information about each paper. Look at the table carefully and make yourself familiar with the unique features of each paper.

Examination	Papers 1 and 2
types of possible items	• multiple choice • short response items (single-word, sentences or paragraphs) • calculations using algorithms • interpreting graphs, tables or diagrams • responding to unseen data and/or stimulus • extended response (300–350 words or equivalent).
syllabus coverage	• Units 3 & 4
assessment objectives	• describe and explain • apply understanding • analyse evidence • interpret evidence and draw conclusions
conditions	• reading time: 10 minutes • writing time: 90 minutes
equipment	• approved graphics calculator permitted • unseen data
marks	• 50% of total assessment

Careful and thorough preparation for the examination may help reduce some of the anxiety and stress you may experience in the lead-up to and during this assessment. Many students feel that their performance in the examination, as well as all assessments, is what will define them and determine their future career paths.

Remember, no matter how you feel about your performance, there is nothing you can do to change it. If you have not achieved the score that will gain you entrance into the course for your preferred career path, consider your options. Investigate different pathways to move towards your goal. Consider different courses. Seek advice from teachers, especially your careers teacher. Sometimes the path to achieve a career goal is less direct.

Engage in positive self-talk.

It is very common and easy for students to become totally consumed by the demands and pressures of their final year of school. But remember, there is life beyond Year 12!

This guide provides you with practical suggestions and support to prepare for the examination. The guide takes a year-long approach to your preparation that includes:

1 Understanding the course: The course requirements and resources available for support
2 Consolidating learning: Effective learning routines and strategies for use throughout the year
3 Revising and practising: Study practices and strategies in the weeks leading up to the examination.
4 Sitting the exam: Strategies and hints for tackling the examination
5 After the exam: Some considerations

1.16 Examination preparation

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- prepare yourself to successfully complete the examination.

As you work through the QCAA Biology syllabus, it is vital that you take very good notes from classwork and homework. These notes will be the foundation of your revision and summary notes. They should:

- be thorough, covering all aspects of the syllabus
- focus on key concepts
- be balanced and weighted so larger topics are given greater emphasis than minor topics
- include examples and applications
- include definitions of key terms
- include questions and answers (that have been checked)
- be legible, so you can easily read them.

CONSOLIDATING LEARNING

Begin studying early. It is easy to procrastinate and then find yourself rushing to revise and study at the last minute. That is not a recipe for success. Learning is more effective if it is spread over a long period instead of a last-minute cram.

Learning requires discipline over the duration of the course. Repetitive and frequent revision of the course material helps to reinforce and deepen understanding and helps embed learning in long-term memory. Studying and revising can be overwhelming. Revise manageable chunks by dividing your work into smaller sections or topics.

During the year, get into the habit of revising completed sections of the course. The revision may be done, for example, at the end of a week, a topic, a chapter or at intervals that best suit you. Revise work from earlier in the year on a regular basis. It is easy to forget information if you don't keep reminding yourself.

There are a number of revision strategies you will use. These are outlined below. Identify your own study strengths to determine what works best for you and make effective use of these strengths.

Some effective learning routines and strategies for use throughout the year are described below.

Time-management is important.

- Use your study time effectively.
- Use your leisure time effectively.

Consult your teacher

Your teacher is your biology expert. He or she is an excellent resource and should be the first person to consult for support and help. Seek help during classes if you are struggling to understand concepts. If you encounter problems out of class time, it is a good idea to write down areas that you are struggling with, and consult your teacher later. Remember to pinpoint specific concepts or questions. Ask your teacher to re-explain concepts and ask for more material and/or questions for practice.

Make flash cards

Make your own flash cards, as this consolidates your learning. Each card is dedicated to a particular idea that could include:

- definition—key term on one side and the definition on the back
- diagram—diagram on one side and a brief explanation on the back
- equation/formula—formula on one side and a worked example on the back
- graph—graph on one side and a summary of results on the back
- question—question on one side and the answer on the back.

Use the cards to test yourself. You can get friends or family to test you. Periodically reading the cards reinforces learning and entrenches concepts in long-term memory (Figure 1.16.1). Flash cards are an excellent tool for subject matter that must be remembered.

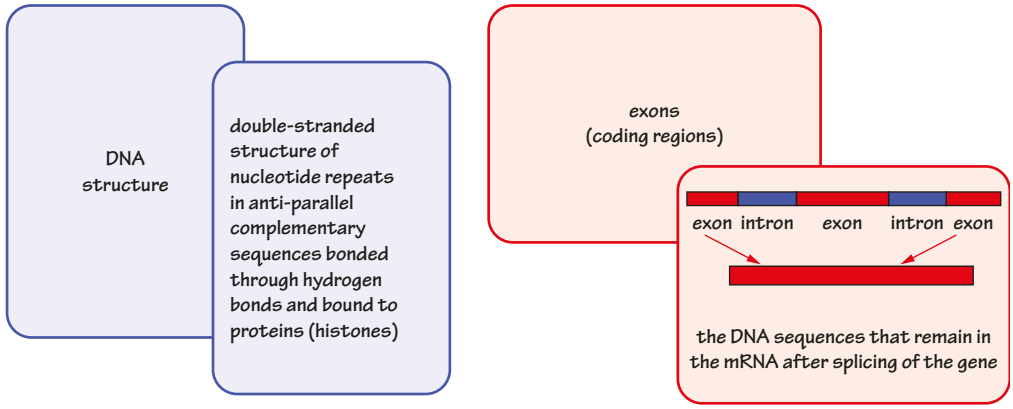


FIGURE 1.16.1 Flash cards are excellent tools for remembering and testing yourself on factual material.

Trigger words

Write down the key words associated with a topic or theme. Trigger words are useful in helping to generate other related words and ideas. This approach is helpful in preparing quick drafts to questions and concept maps to summarise whole topics.

Diagrams

Diagrams are useful memory triggers. Draw first, explain second!

Test yourself

Complete as many questions as possible, to test your learning. Handwrite (not type) your answers. Writing is more valuable practice than just thinking about your answer. Writing is also a good way to memorise material. Remember, you will need to be able to write for 90 minutes in the allocated writing time in the examination and your writing must remain legible.

Summarise your notes

Consider your preferred learning style and take advantage of it to make revision notes. Summaries help you identify the essential concepts and reinforce your learning. Study notes are most effective when they are:

- clear
- to the point
- uncluttered.

Some techniques you may apply to make summaries include:

- highlighting or underlining important points as you read your notes or text
- creating headings or appropriate structures to organise dot points under
- using diagrams to summarise concepts
- using tables to summarise comparisons (e.g. causes/effects, for/against)
- creating summary sheets
- using boxes, circles and colour to highlight points on your summary sheets
- creating ways to remember key points, for example, mnemonic devices like 'King Phillip Came Over From Great Spain' as a device for remembering taxonomy order (Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species)
- compiling a glossary of new terms in your own words, not taken straight from the textbook
- using scatter diagrams, mind maps or concept maps to organise key information. (Figure 1.16.2).

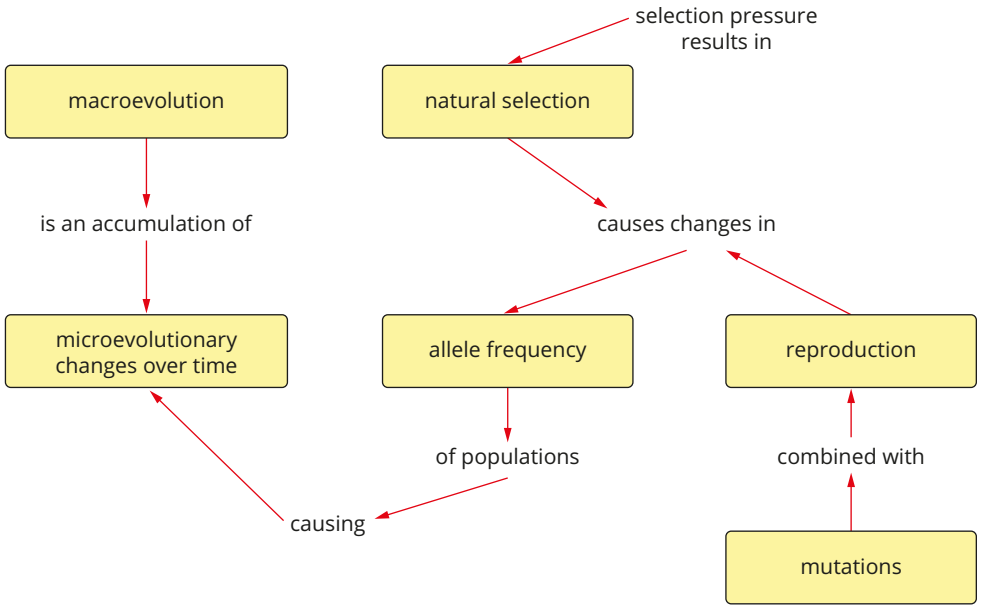


FIGURE 1.16.2 Visual representations in the form of concept maps are effective revision and study tools.

Check there are no gaps in your summary notes. Use chapter and module openers and module review summaries as a checklist for the key ideas in a chapter.

- Tick those with which you feel confident.
- Highlight those you need to revisit.
- Re-read relevant sections of text.
- Rewrite material (e.g. notes, definitions, equations) that you need to consolidate.

Form a study group

Form a small study group with friends. The group can meet face-to-face or online. Discuss and explain key points to each other. If you are struggling with a difficult concept, try to teach it to a friend. Ask a friend to teach you. You cannot explain something to another person until you have it clear in your own mind. Teaching and explaining to someone else makes it clearer to you.

Practise

Do lots of practice questions. Write down your answers so that you get practice expressing yourself. Verbalising or thinking the answer is not the same, and your responses may not always come out in writing how you want them to unless you have plenty of practice.

REVISING AND PRACTISING

In the month and weeks leading up to the exam, most if not all of the course will have been taught. Your focus should now shift to studying in preparation for the exam. You will be at an advantage if you have taken comprehensive notes throughout the year, made revision summaries, completed practice questions and generally made every effort to advance your understanding and skills.

Prepare a study timetable

Allocate time to study all the subject matter. The timetable may include daily study time across all your subjects and may specify sections of each subject to be covered daily. While you may not stick strictly to the timetable, it is a guide to fitting everything in and allocating equal time to different subjects.

Prepare summary notes

You may have been making summary notes of sections of subject matter throughout the year. It is now time to consolidate all your learning across Units 3 and 4. Remember that different topics link with each other and it is only when a whole topic or unit is completed that these links become apparent. The summary notes you have previously prepared can be collated and serve as the foundation of one summary of the Biology course.

Create a revision summary

List all the topics and subtopics in the syllabus. The chapter opening pages of your student book will guide you. You might choose to prepare a separate table for each topic, and use it to write your revision notes. Table 1.16.1 demonstrates the table that may be created for summarising and revising Topic 1 of Unit 3 (it is only a partial example). It allows for key points, definitions and formula for each subtopic. While this layout may suit some students, it may not suit you. Vary the labels and organisation to best suit you. For example, you may choose to include a column for worked examples and/or sample questions.

TABLE 1.16.1 Sample revision notes

Revision notes for Unit 3: Biodiversity and the interconnectedness of life		
Topic 1: Describing biodiversity Subject matter: Biodiversity	**Key points**	**Key terms and definitions/diagrams**
Biodiversity—recognise it includes diversity of species and ecosystems.	Diversity of species includes variations within a species and also variation between species. Diversity of ecosystems is vast and includes differences in abiotic factors (e.g. climate, substrate, size/depth of area) and biotic factors within ecosystems and also differences between ecosystems.	diversity species ecosystem abiotic biotic
Determine diversity by: a species richness b evenness c percentage cover d percentage frequency e Simpson's diversity index.	Species richness specifies the number of different species in an area. It provides one descriptive element about the diversity of an ecosystem. Evenness describes the abundance of each species in an area relative to all the other species. It provides one descriptive element about the diversity of an ecosystem.	species richness evenness
Species diversity index to compare ecosystems across space and time: a species interactions b abiotic factors.		
Explain how environment limits distribution and abundance of species in an ecosystem.		

Skim through your student book and class notes. Identify key sections and terms. These can be added to your revision chart, for later elaboration.

Re-read all your class notes carefully for a section of work. Once you feel you understand and remember them, put the notes aside. Without referring back to the notes, start filling in the revision chart. You may need to keep referring back to your notes. Each rereading of the notes will reinforce your learning. Create a manual, not electronic, revision chart. The examination is handwritten, so it is a good idea to practise handwriting your notes as preparation.

Refer to additional resources if you do not understand anything. This might mean checking the student book, asking your teacher, researching online or discussing with classmates. You can keep track of problem areas by writing questions in the margin of the revision table, highlighting parts of the revision notes you do not fully understand or keeping a separate queries sheet.

Dot point notes, visuals, tables, graphs are all useful ways of summarising information. Use these in your revision table as appropriate. It is often easier to bring to mind information in these forms in an exam, rather than try to visualise a large block of text. If you are a visual learner, it may be helpful to colour-code your notes to emphasise different concepts. Rewriting notes, whether in note or visual form, helps you remember them.

Learn the subject matter. In preparing the revision table, read and re-read the subject matter so that your understanding continually improves. Practice rewriting the revision table without referring to the original one. Try making a summary of the revision table so that you are focusing on the essential concepts. Discuss concepts with classmates to clarify your understanding. Give the revision chart to a family member and ask them to test you.

Practise, practise, practise. Complete as many test and exam papers as you can. Sitting an exam requires skill and the more practice you have in advance, the more prepared you will be. Take practice exams in the time limit allocated. Handwrite the answers to practise working quickly, writing your ideas legibly and expressing yourself clearly. This will show you what you know, but more importantly, what you do not know and need to revise again.

The examination consists of two papers. For each paper, make sure you know:

- the time allowance
- the date and time of the examination
- the type of questions to expect
- equipment and materials you are permitted to take in
- materials and equipment to be supplied for use in the examination.

Be prepared. Do not cram the night before the examination. Re-read your revision table to refresh the subject matter that you have been learning over the last few weeks.

SITTING THE EXAMINATION

Be prepared. Double-check the date, time and place of the examination. Arrive with plenty of time to spare. Running late will only stress you before you even get into the examination room!

Remain calm and focused as you enter the room. At this stage, it is too late to worry about your application through the year or your examination preparation.

Here are some strategies that will improve your chances of doing well.

Perusal or reading time

Every examination allocates time for candidates to look over the paper. You are not permitted to pick up pens or pencils at all during this time, not even to write your identification on the answer booklet. Use this time wisely. It is valuable for you to get an overview of the paper.

- Check through all of the paper quickly.
- Carefully read and re-read the instructions.
- Plan how much time you will allocate to different sections of the paper.
- Start reading questions and answer them mentally.
- If a question is difficult, do not dwell on it. Move on to the next one. Sometimes another question can give clues to ones that initially appeared difficult.
- Carefully read each question. In your head, translate into your own words what you are being asked to do.
- Consider the marks allocated to each question. Plan to spend more time on questions that carry more marks.

Writing time

Start writing as soon as you are permitted.

Read the questions carefully

If you find it helpful, underline key words in the question. This can be a useful strategy for identifying the focus of the question and giving direction about what is required in your answer. For questions with multiple parts, read the whole question before starting to write your answer. This gives an overview of the question. Sometimes the information in one part of a question will provide a hint for another part. Avoid repeating the same information in different parts of the same question.

Interpret the questions

Read questions carefully and look for key verbs like 'explain', 'compare', and 'determine' that tell you what you are expected to do. It may be helpful to highlight or underline key parts of questions. The questions in module, chapter and unit reviews of your student and activity books have been written using verbs from the syllabus. The practice examinations you did also developed your skills in analysing questions. You should be familiar with the style of questioning and had practice at interpreting questions. Make sure your answers address all parts of the question. Use scientific terms.

Show your working

Show ALL the steps you took to reach your solution for questions involving calculations. While there may be an error in one part of the calculation, you may still score some marks for using the correct method. Remember to always include the correct units in answers. Remember to correctly label all graphs (x and y axes, title, units). If you do work that you think is incorrect, simply put an 'X' through it, instead of spending time erasing it completely.

Working with data

Read all the information carefully so that your answers are accurate. For graphs, read the labels on the axes and understand the relationship shown by the graph. The title of the graph will assist with this. Use a ruler for reading graphical data accurately.

When looking at tables, read the headings of rows and columns carefully so you understand the content of the table.

Always include the proper units for each number where appropriate. If you keep track of units as you perform your calculations, it can help ensure that you express answers in terms of the proper units. Depending on the question, it is often possible to lose points if the units are wrong or are missing from the answer.

Answer all the questions

An unanswered question cannot possibly earn you any marks. It is better to take an intelligent guess that might earn some marks than not to attempt an answer. When you have completed an answer, re-read the question. Check that you have addressed the question. An answer that includes correct information but does not answer the question will not earn any marks.

Many free-response questions are divided into parts, such as a, b, c and d, with each part calling for a different response. Credit for each part is awarded independently, so you should attempt to solve each part. For example, you may receive no credit for your answer to part a, but still receive full credit for part b, c or d. If the answer to a later part of a question depends on the answer to an earlier part, you may still be able to receive full credit for the later part, even if your earlier answer is wrong.

Use the answer sheet

Some examinations will require you to complete a separate answer sheet for the multiple choice questions. If your examination does, make sure that you mark your answers on this sheet, not only on the question paper. If you make an error, erase it carefully before filling in a new answer.

Answer writing tips

Do not rewrite the stem of a question to answer the question. You can deceive yourself by feeling you've filled up the allocated space, without actually answering the question.

Sometimes an answer is simply a one-word answer or a numerical reading from a graph. Where possible, use standard phrases that neatly encapsulate meanings rather than wordy explanations. For example:

- carrying capacity
- osmotic pressure
- genetic diversity
- phenotype.

Don't give extra information. This is unnecessary and can lead to irrelevant or even contradictory information. It is also an inefficient use of your time.

If you aren't sure of the answer, don't write two possible responses. For example:

Question	Sample answer	Result
Name the stored form of glucose in animal cells.	Glycogen or starch.	No score

Answer the question that is being asked. Providing accurate information that doesn't address the question doesn't attract a score. For example:

Question	Sample answer	Result
Identify the mutation in the genetic sequence TTA GTC CAT GCC from the original sequence of TTA AGT CCA TGC.	Mutations in genetic sequences change the code and cause errors.	No score
	Insertion point mutation.	Scores

If a question asks for an explanation, a one-word answer will not be enough. For example:

Question	Sample answer	Result
Explain why you agree or disagree with the following statement: A homozygous cross between a dominant allele genotype and recessive allele genotype produces only heterozygous hybrids.	Agree.	No score
	Agree. Due to the law of segregation, each parent will pass on a single allele resulting in the filial generation receiving one copy of the allele from each parent. As the parents are homozygous for different alleles, the filial generation will be heterozygous.	Scores

Be clear about what you mean. There should be no room for interpretation. For example, 'adaptation' could mean change in allele frequencies in a population or the physiological response in homeostasis.

Use the number of lines provided as a guide about how much to write. Use the number of marks allocated to a question or part of a question as a guide about how many points to make. Consider whether you can add more details, clearer explanations or make corrections to answers.

Pay attention to your spelling.

Never leave blanks!

Easier questions could be completed first.

Multiple choice questions are designed to make you select between very similar items. Read all options carefully. If you are unsure which choice is correct, identify choices that are wrong. Answer every multiple choice question, even if all you can do is guess. Answer each multiple choice question before you move on to the next one. You can mark a question to come back to later if you have time but you should still select an answer before you move on. You should allow just over a minute for each question. If the question takes longer than that, take a guess and move on.

Difficult questions

If you find a question particularly difficult, skip it. Come back to it later if you have time. Don't use a 'scattershot' or 'laundry list' approach, like writing answers with as many equations or lists of terms as you can, hoping that the correct one will be among them so that you get partial credit. If a question asks for two or three examples or equations, only the first two or three examples will be scored.

Don't skip a question. Have a guess—no marks will be deducted for an incorrect answer but you may pick up marks for a partial response. Also, if you're unsure of a multiple choice answer, try to eliminate one or more of the options and then have a guess. You may get lucky!

Mental blocks

Sometimes when you are under pressure, your mind can go blank. The information is there but is temporarily unavailable to you. Take a few deep, slow breaths. Go back to the question. Try to visualise your notes on the revision tables you created. If this does not work, move onto another question and return to the question later.

Recheck your answers

If you have time, return to questions that you are unsure of. There is no need to check all answers if you run out of time. Re-read each question when you have finished answering. This will help you to be sure that you have addressed every part of the question and haven't made an error that could change the meaning of your response.

Stay for the allocated time

Never leave the examination early. Examinations are generally designed to take the full writing time allocated to them. If you finish early, recheck for anything you may have missed. Check for blank pages and on both sides of the page to make sure you haven't missed anything. Count the number of questions you have answered against the number listed on the front of the exam.

Ignore students who leave early. It does not mean they performed well. It often indicates they did not study enough and do not know the subject matter.

Remember:

- You can only be asked about information that is included in this course.
- The exam is your opportunity to showcase your knowledge of this subject.

AFTER THE EXAMINATION

Once you have left the examination room, avoid talking to other candidates. Everyone has an opinion of their own personal performance. Listening to others can be unsettling. Whether you feel you performed well or are disappointed with your performance, it is now beyond your control. Remain calm. It is one examination result, in one subject, in one year of your life. No matter what happens, there are plenty of career pathways to pursue.

UNIT 3 Biodiversity and the interconnectedness of life

TOPIC 1 Describing biodiversity

TOPIC 2 Ecosystem dynamics

Unit 3 objectives

Students will:

1. describe and explain biodiversity and ecosystem dynamics
2. apply understanding of biodiversity and ecosystem dynamics
3. analyse evidence about biodiversity and ecosystem dynamics
4. interpret evidence about biodiversity and ecosystem dynamics
5. investigate phenomena associated with biodiversity and ecosystem dynamics
6. evaluate processes, claims and conclusions about biodiversity and ecosystem dynamics
7. communicate understandings, findings, arguments and conclusions about biodiversity and ecosystem dynamics.

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CHAPTER 02

Classification processes

Classification is the sorting of biotic and abiotic factors around us into groups based on shared or similar characteristics. The power in classification is not merely that it helps scientists make sense of the physical universe, but that it enables them to see the interrelationships and interdependencies between different groups. Having a common language of classification also allows scientists to share their knowledge and discoveries with the wider scientific community.

Classification was once based on where an organism lived and what it looked like. However, with the advent of modern technology in the form of DNA sequencing and computer algorithms, scientists are able to ascertain relationships between organisms to a greater degree than used to be thought possible. That knowledge can be applied to manage and protect the biodiversity in the ecosystems around us.

In this chapter, you will learn that the classification of organisms can be based on a range of characteristics, including their physical appearance, their method of reproduction, accumulated variations in the molecular sequences of both genes and proteins, or according to their species interaction. You will also understand that in order to effectively manage the continued survival of organisms, we must also understand the ecosystems in which they live. Finally, you will learn that ecosystems are comprised of varied habitats, all of which need to be classified and managed in their own right.

Syllabus subject matter

WS 3.1.1

Topic 1 • Describing biodiversity

CLASSIFICATION PROCESSES

- recognise that biological classification can be hierarchical and based on different levels of similarity of physical features, methods of reproduction and molecular sequences
- describe the classification systems for
  - similarity of physical features (the Linnaean system)
  - methods of reproduction (asexual, sexual—*K* and *r* selection)
  - molecular sequences (molecular phylogeny—also called cladistics)
- define the term clade
- recall that common assumptions of cladistics include a common ancestry, bifurcation and physical change
- interpret cladograms to infer the evolutionary relatedness between groups of organisms
- analyse data from molecular sequences to infer species evolutionary relatedness
- recognise the need for multiple definitions of species
- identify one example of an interspecific hybrid that does not produce fertile offspring (e.g. mule, *Equus mulus*)

- explain the classification of organisms according to the following species interactions: predation, competition, symbiosis and disease
- understand that ecosystems are composed of varied habitats (microhabitat to ecoregion)
- interpret data to classify and name an ecosystem
- explain how the process of classifying ecosystems is an important step towards effective ecosystem management (consider old-growth forests, productive soils and coral reefs)
- describe the process of stratified sampling in terms of
  - purpose (estimating population, density, distribution, environmental gradients and profiles, zonation, stratification)
  - site selection
  - choice of ecological surveying technique (quadrats, transects)
  - minimising bias (size and number of samples, random-number generators, counting criteria, calibrating equipment and noting associated precision)
  - methods of data presentation and analysis.

SCIENCE AS A HUMAN ENDEAVOUR

Technology as a tool to measure, analyse and monitor biodiversity: Advances in remote sensing radar imagery and satellite tracking in real time have enabled scientists to measure and monitor populations, and play a significant role in surveying and monitoring large or inaccessible ecosystems.

MANDATORY PRACTICAL 1

Use the process of stratified sampling to collect and analyse primary biotic and abiotic field data to classify an ecosystem.

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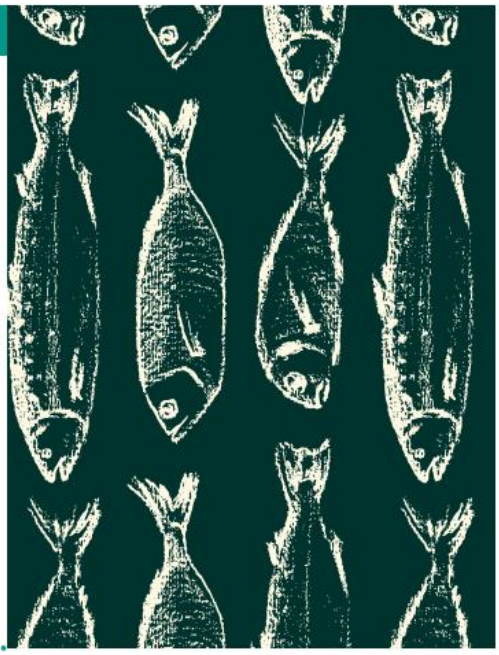
2.1 Classification

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recognise that biological classification can be hierarchical and based on different levels of similarity of physical features, methods of reproduction and molecular sequences
- describe the classification systems for:
  - similarity of physical features (the Linnaean system)
  - methods of reproduction (*K* and *r* selection)
  - molecular sequences (molecular phylogeny)
- recognise the need for multiple definitions of species
- identify one example of an interspecific organism that does not produce fertile offspring
- explain the classification of organisms according to the following species interactions: predation, competition, symbiosis and disease
- interpret data to classify and name an ecosystem
- understand that ecosystems are composed of varied habitats (microhabitat to ecoregion).



Classification is the systematic and rational grouping of both organisms and ecosystems in such a manner that shows the biological relationships between them. Worldwide standardisation is achieved through organisations such as International Commission on Zoological Nomenclature, which means that every identified organism will be recognised by the same scientific name around the world.

Every ecosystem can be classified by the same discriminators so that we know that a tropical rainforest in Costa Rica is the same type of ecosystem as a tropical rainforest in the Congo Basin. Even though the plant and animal **species** may differ, the ecosystems function and are structured in the same way. In the same manner, using complete and published anatomical descriptions, every known species of animal, plant, fungi, protist and **bacteria** has been given a unique scientific classification that is accepted and used by all scientists around the world.

IMPORTANCE OF CLASSIFICATION

Biologists spend considerable time classifying organisms by carefully examining them for features in common, grouping them and naming the groups. Classification is not a new practice. Indigenous people around the world also have names for the organisms that they use or need to recognise. For example, the mulga snake (*Pseudechis australis*) is also known as kulipirri by the Ngarla people in Western Australia and as the king brown snake by people living in Queensland (Figure 2.1.1). Classifying organisms into groups and categories is significant to understanding biological relationships.

Taxonomy is the scientific discipline concerned with the naming and classifying of organisms. Taxonomists place organisms into groups known as taxa (singular: **taxon**) and give the organisms unique scientific names. Taxa can be at multiple levels, and include species, genera (singular: **genus**) and families. For example, *Aptenodytes forsteri* (the emperor penguin) is a species taxon. It belongs to the genus taxon *Aptenodytes* (the great penguins), which in turn belongs to the **family** taxon Spheniscidae (the penguins).

FIGURE 2.1.1 *Pseudechis australis* is the heaviest venomous snake in Australia.

FIGURE 2.1.2 A coral reef in the Red Sea, showing the large diversity of organisms.

FIGURE 2.1.3 The mucus secreted by mushroom coral is an effective sunscreen.

The scientific system of placing groups of organisms into taxa is called **biological classification**. This system enables us to understand the relationships, evolution and diversity of living things. By applying an internationally recognised system of classification, you can quickly obtain information about a particular organism. You can tell, for example, whether it is a mammal or a fish, or a flowering plant or a fern. Because every taxon in a **kingdom** has a unique name, scientists know exactly what organism they are talking about when they use that name.

Recording biological diversity

The biosphere is the region of the Earth inhabited by living things. It includes seas, lakes, rivers, soil and the lower atmosphere. Humans share the biosphere with millions of other species (Figure 2.1.2). This biological diversity, or biodiversity, is very important to our wellbeing because we use other species as sources of food, shelter and medicines. We are still discovering possible uses for many species. For example, mushroom corals (Figure 2.1.3) are exposed to the sun's radiation during low tides. Compounds that they secrete in their mucus act as an effective sunscreen, preventing them from damage during this time. Knowledge of these corals and the use of their compounds may help to protect people from skin cancer.

It is estimated that there are at least 8.7 million species of eukaryotes in the world today, but only 1.2 million of them have been named. This means that about 7.5 million species of eukaryotes have yet to be described. Only by recording biodiversity will we know what organisms exist, at what rate they are becoming extinct, which habitats are most endangered and in need of protection or restoration, and which species and habitats can be used safely for the benefit of people.

CLASSIFYING ORGANISMS

Common names are often used to refer to a group of similar organisms that have features in common. However, common names often mean different things to different people and can therefore be confusing. For example, the common name 'tiger' is used both for the feline tiger (*Panthera tigris*) and the Tasmanian tiger (*Thylacinus cynocephalus*). However, one is a placental mammal and the other is an extinct marsupial. They are very different animals indeed (Figure 2.1.4).

Biodiversity is the diversity of all life forms, the genes which they contain, and the ecosystems of which they are a part. You will learn more about biodiversity in Chapter 3.

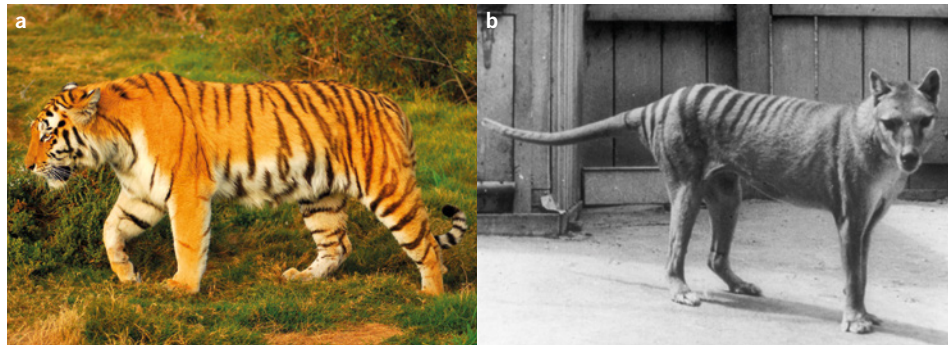


FIGURE 2.1.4 (a) A Siberian tiger (*Panthera tigris altaica*) is very different to (b) the extinct marsupial Tasmanian tiger (*Thylacinus cynocephalus*) even though they share a common name.

Binomial system

To avoid confusion when scientists communicate with each other, organisms are referred to by their scientific names, which are based on Latin. The scientific name of each species consists of two parts and is known as a **binomial**. The system of using a two-part name is called the **binomial system**. The binomial system was developed in the 18th century by Swedish botanist Carl **Linnaeus** (1707–1778).

In a binomial, the first part of the name is a generic name, or the genus. A genus is a group that includes one or more species (Figure 2.1.5).

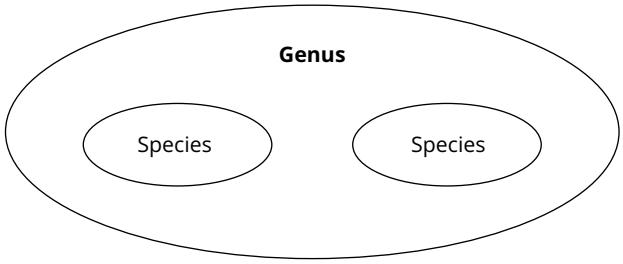


FIGURE 2.1.5 A genus is a larger group that includes one or more species.

The second part of a binomial is the species name (abbreviation 'sp.'). It is a specific descriptive name that is unique for each species within the genus. For example, *Agra grace* and *Agra vation* are the scientific names for two different kinds of beetles. Both species belong to the same genus *Agra* (Figure 2.1.6). To indicate you are writing about all species within the genus *Agra*, you would write *Agra* spp.

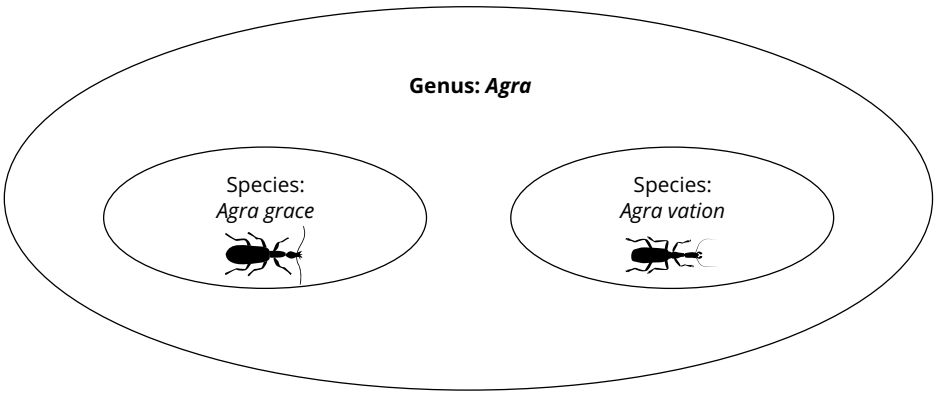


FIGURE 2.1.6 The species *Agra grace* and *Agra vation* belong to the same genus *Agra*.

The genus name always begins with a capital letter and the species name always begins with a lower-case letter, even if it is named after a person or a place. When scientific names are typed they should be italicised, but they can be underlined if handwritten.

The binomial system for naming species is concise and precise. Its international acceptance resulted in a great improvement in communication within the scientific community.

Naming species

Since Linnaeus introduced binomial classification, the species has been the basic unit in the classification of organisms. Traditionally, differences in the morphology (form and structure) of organisms have been used to divide organisms into species. However, not all biologists agree on the definition of 'species'.

In the 1940s, biologist Ernst Mayr introduced a single concept of species for all organisms. This was based on the concept of **biological species**. According to this concept, members of a single species breed and produce offspring in the wild. Members of different species generally do not breed in the wild.

The term 'species' means a group of organisms that resemble each other more than they resemble members of other groups. A biological species refers to individuals that can breed among themselves and produce fertile offspring.

FIGURE 2.1.7 House sparrows freely breed with one another but not with other species.

For example, house sparrows breed with one another (Figure 2.1.7), but not with other species. However, species of many organisms do not reproduce sexually (for example, bacteria, some insects and many protists undergo both sexual and asexual reproduction), so Mayr's criterion of a biological species (that can breed among themselves and produce fertile offspring) is not relevant for those organisms.

There are more than 20 different definitions for the term 'species' used in the scientific community today. Apart from Mayr's definition, the other most commonly used definitions are the ecological and genetic definitions. The ecological form defines a species by the resources that a particular group of organisms uses and depends on in the specialised ecological niche that the group occupies. Different forms of this definition classify organisms by how they reproduce, their life cycles, or how they interact with other species within their ecosystem. The genetic definition of species utilises both the inherited genetic traits of an organism and the amount of genetic variation in the populations of that organism. This is the definition that is used in molecular phylogeny, or cladistics.

Subspecies

Some species show variation in **form** between populations across their total geographic range. The differences may be slight, or a series of subtly different forms may spread along a geographic range. In these cases, taxonomists recognise **subspecies** to convey this information. Subspecies usually have different geographical distributions from each other. For example, the tiger (*Panthera tigris*) was once classified into nine subspecies based on geographic distribution. More recent studies have used a combined approach of collecting and analysing molecular, **morphological** (physical features) and ecological (which includes geographical distribution) data. Their findings support the recognition of two distinct subspecies only—the continental Asian tigers and the Sunda tigers (Figure 2.1.8).

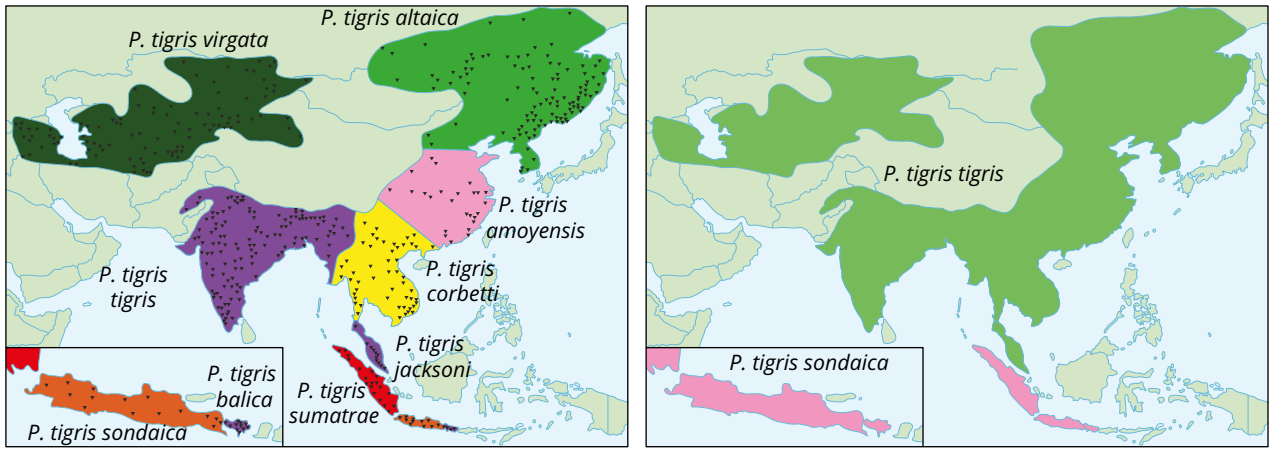


FIGURE 2.1.8 As a result of DNA analysis and morphological data, the current nine subspecies of tiger have been deemed to only have enough variation to be considered two subspecies – *Panthera tigris tigris* and *Panthera tigris sondaica*. The black triangles indicate where specimens were collected.

FIGURE 2.1.9 DNA testing is required to determine whether this plant is a pure *Eucalyptus melliodora* (yellow box) or an interspecific species between *Eucalyptus melliodora* and *Eucalyptus moluccana* (grey box).

Interspecific hybrids

Although members of different biological animal species do not generally interbreed in nature, they can do so under certain circumstances. The exceptions are known as **interspecific hybrids**, which can occur when closely related species are kept together in game parks or zoos (rather than natural habitats). For example, the lion and tiger do not usually mate in the wild, but interspecific hybrids of these two species have been produced in captivity: the liger (the offspring of a male lion and a female tiger) and the tigon (the offspring of a male tiger and a female lion).

However, it is more common for certain plant groups to interbreed in nature. One of the main difficulties in identifying members of the genera *Eucalyptus*, *Corymbia*, *Callistemon* and *Melaleuca* in the wild is due to their being readily able to crossbreed with other species of the same genus (Figure 2.1.9).

The best-known animal interspecific hybrid is the mule, which is the hybrid offspring from a mating between a male donkey and a female horse (Figure 2.1.10). Most mules are sterile (unable to produce offspring). The wolphin is a very rare animal interspecific hybrid that is the result of a mating between a male false killer whale and a female bottlenose dolphin. In 2014, a wild-bred, injured wolphin was cared for at Sea World on the Gold Coast (Figure 2.1.11). However, at Sea Life Park in Hawaii, a captivity-bred female wolphin successfully mated with a male bottlenose dolphin to produce two healthy female calves, demonstrating that at least one interspecific hybrid wolphin was fertile.

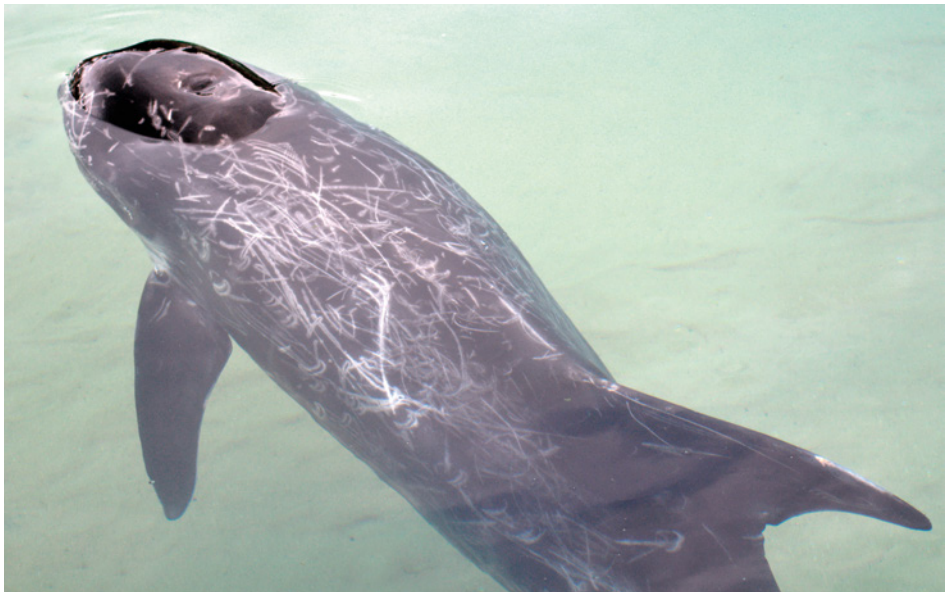


FIGURE 2.1.11 A wolphin at Sea World on the Gold Coast.

FIGURE 2.1.10 A mule (left) and a horse (right). A mule is an interspecific hybrid between a male donkey and a female horse.

FIGURE 2.1.12 The house sparrow (*Passer domesticus*).

FIGURE 2.1.13 The genus *Fringilla* includes three species: the common chaffinch (*Fringilla coelebs*) shown here, the blue chaffinch (*Fringilla teydea*) and the brambling (*Fringilla montifringilla*).

Writing subspecies and varieties

Subspecies are recognised for animals, but varieties are not. The subspecies name is written immediately after the species name, as in the continental Asian tigers (*Panther tigris tigris*) and the Sunda tigers (*Panther tigris sondaica*).

For plants, both subspecies and varieties are recognised. They are written using the abbreviations 'subsp.' and 'var.' For example, *Zieria arborescens* subsp. *decurrens* is a subspecies of the forest zieria, and *Zieria aspalathoides* var. *intermedia* is a **variety** of the hairy zieria.

If you are handwriting names, you can use an underline instead of italics.

Scientific names can change

Taxonomy is a field that is constantly evolving. Scientific names of species are determined by the first biologists to publish a description of the organism. However, names can change when scientific understandings of animal species and their evolutionary relationships change, or earlier published manuscripts are uncovered.

Sometimes a species might have been placed within a genus (or other group) of species to which it is not most closely related. For example, the house sparrow (Figure 2.1.12) was initially named *Fringilla domestica* by Linnaeus in 1758 because it has similar characteristics to other species in the genus *Fringilla*. Shortly afterwards, the genus *Fringilla* was restricted to finches of the Old World, such as the common chaffinch *Fringilla coelebs* (Figure 2.1.13). In 1760, Mathurin Jacques Brisson created the genus *Passer* for sparrows, and the scientific name of the house sparrow became *Passer domesticus*.

If DNA or other new evidence indicates that a mistake has occurred, a species may be reclassified to more accurately reflect its evolutionary relationships with other species.

FIGURE 2.1.14 The North American bobcat (*Lynx rufus*).

FIGURE 2.1.15 The laughing kookaburra (*Dacelo novaeguineae*).

The genus *Felis* once included all small cat species. The genus has now been split into several genera to show the evolutionary differences between the species. For example, the bobcat of North America was once known by the scientific name *Felis rufus* but is now known as *Lynx rufus* (Figure 2.1.14).

The laughing kookaburra of Australia (Figure 2.1.15) was first described by Pieter Boddaert (1730–1795) in December 1783 and commonly referred to in the scientific community as *Dacelo gigas*. Nearly 200 years later, it was established that another naturalist, Johann Hermann (1738–1800), had published using the name of *Dacelo novaeguineae* in September 1783. According to the rules set by the International Commission on Zoological Nomenclature, the name of the laughing kookaburra is now officially recognised as *Dacelo novaeguineae* (Hermann).

Biological classification

As well as developing the binomial system, Linnaeus also grouped similar organisms into a **hierarchy**. The hierarchy places species into groups that are increasingly more inclusive. This system is known as the **Linnaean system**. The first grouping is built into the binomial, where species with the most similar physical features (or characteristics) are grouped together into the same genus.

For example, sugar gliders (*Petaurus breviceps*) are small possums with a relatively short head, grey fur and a membrane attached to their sides and limbs that allows them to glide from tree to tree (Figure 2.1.16). Sugar gliders belong to the same genus, *Petaurus*, as yellow-bellied gliders (*Petaurus australis*). Yellow-bellied gliders, as their name suggests, have yellowish fur on their belly (Figure 2.1.17). Although not identical to each other, sugar gliders and yellow-bellied gliders are so similar in size, shape and ability to glide that they can be put together to form a larger group of gliders, a genus.

FIGURE 2.1.16 A sugar glider (*Petaurus breviceps*) with limbs outstretched showing the membrane attached to its sides.

FIGURE 2.1.17 Yellow-bellied gliders (*Petaurus australis*) are similar in form and structure to sugar gliders, but features such as fur colour distinguish them as a different species.

Similar genera are grouped together into a family, families are grouped into an **order**, orders are grouped into a **class**, classes are grouped into a **phylum** (plural: phyla), phyla are grouped into a kingdom, and kingdoms are grouped into a **domain** (Figure 2.1.18). At each level the difference between groups becomes greater. Two species in the same genus will be much more similar than two families in the same order.

The five kingdoms

In the 18th century, Linnaeus classified all living organisms into one of two kingdoms—Animalia and Plantae. Plants and animals certainly appear very different—animals move around and capture food, whereas most plants cannot move around and are green. In modern times, with the development of the techniques of biochemistry, cell biology and electron microscopy, other important and fundamental differences between organisms have been identified. These discoveries have led biologists to recognise other major groups of living organisms that have had to be placed in separate kingdoms. Until recently the most widely accepted system of classification recognised five kingdoms (Figure 2.1.19), which were the largest groupings of organisms:

- Protista (mostly unicellular organisms: eukaryotes)
- Plantae (land plants: eukaryotes)
- Fungi (fungi: eukaryotes)
- Animalia (animals: eukaryotes)
- Monera (bacteria: prokaryotes).

Although the five-kingdom system of classification is sometimes still used, it has been largely replaced by a new system based on DNA evidence.

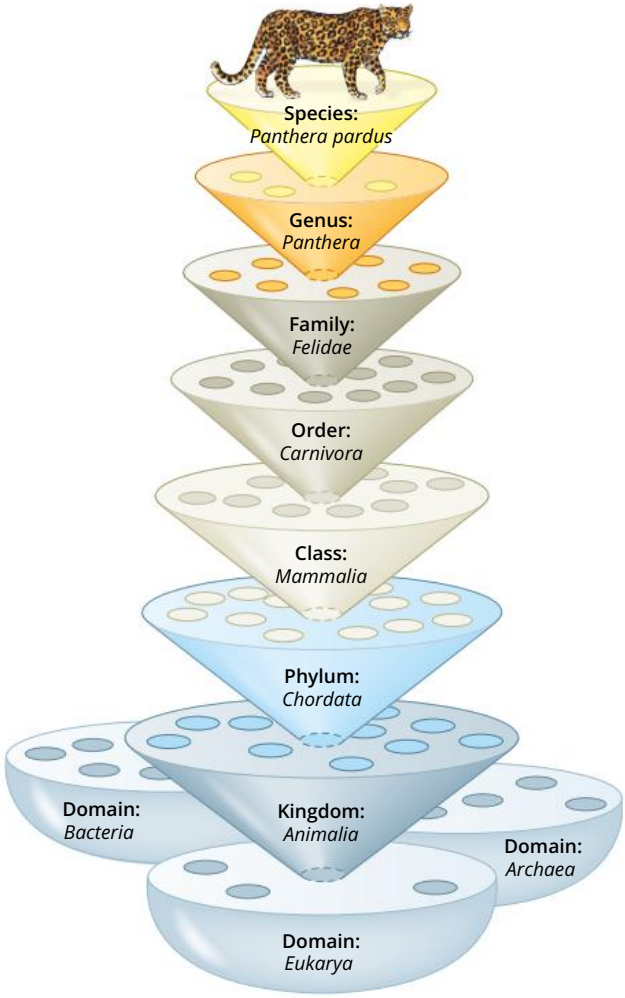


FIGURE 2.1.18 At each level in the Linnaean system, organisms are placed into groups that are more diverse.

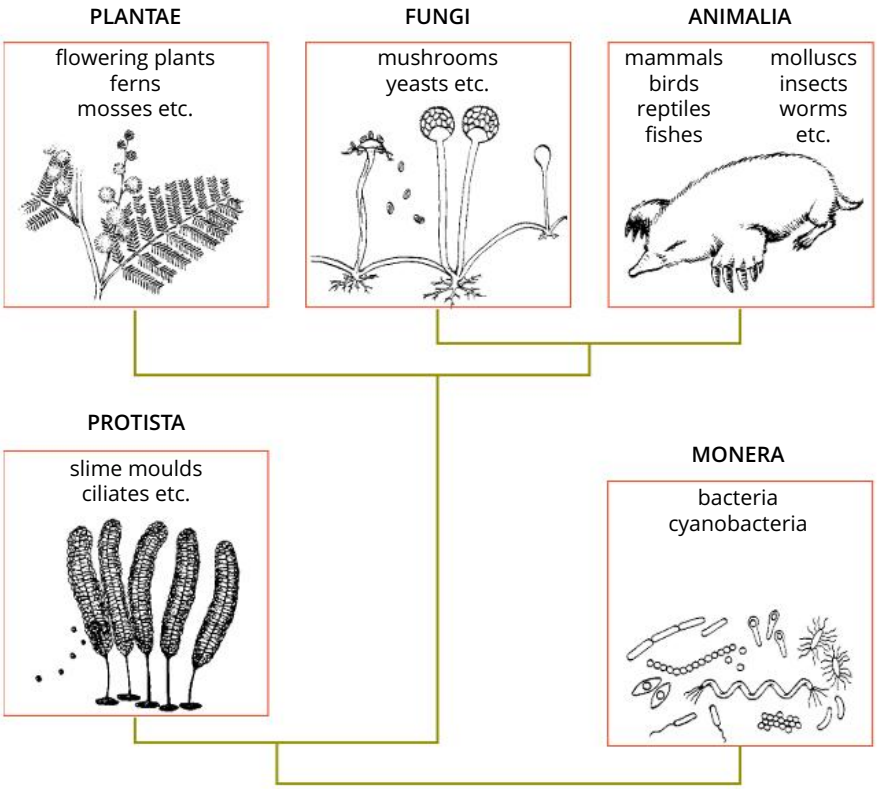


FIGURE 2.1.19 The five kingdoms.

The three domains

In the 1970s, microbiologists Carl Woese and George Fox discovered two distinct **lineages** in bacteria, using DNA techniques that had only recently become possible. Woese went on to develop the theory that there are three major lines of evolution among living things, which he called 'urkingdoms'. Despite initial scepticism from leading biologists, more evidence gradually accumulated to show that Woese's hypothesis was supported, and it developed into an accepted theory. By the 1980s, it had become widely accepted, and is the system of classification that is used today.

The three-domain system infers that there are two major lines of evolution among the bacteria, and that all **eukaryotes** (protists, plants, fungi and animals) are related to one another in another major lineage. The three major lineages are called domains (Figure 2.1.20). The old **prokaryote** kingdom of Bacteria is split into two domains, the Bacteria and Archaea, and the four eukaryote kingdoms are grouped together in the domain Eukarya.

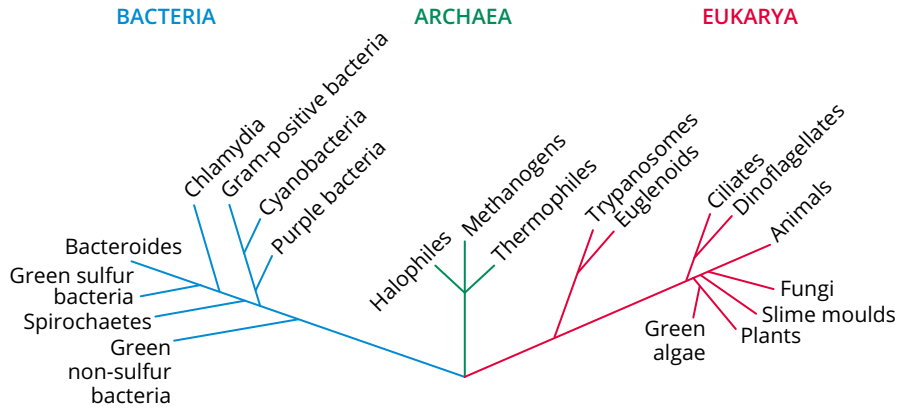


FIGURE 2.1.20 The three domains.

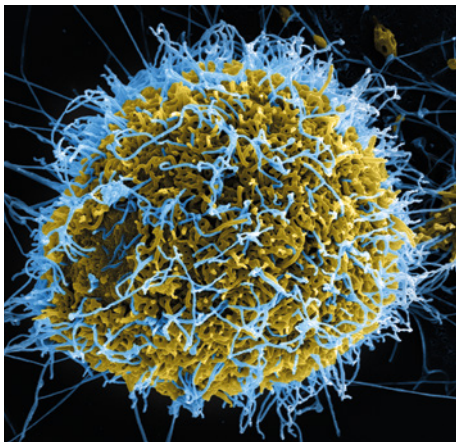


FIGURE 2.1.21 Coloured scanning electron micrograph of Ebola virus particles (shown in blue) on the surface of an infected cell.

Viruses

Viruses (Figure 2.1.21) are peculiar in that they are not classified as living organisms and so fall outside of conventional classification. They do, however, have a major impact on living organisms, as they carry genetic information and must invade living cells in order to reproduce. Viruses themselves have their own separate classification system based on their DNA or RNA, outer protein shell and required host.

Classifying organisms by methods of reproduction

Organisms can also be classified, or grouped, according to the way they reproduce. While sexual reproduction is used by many organisms, asexual reproduction is also quite common in invertebrates, protists, bacteria and some plants and fungi. Some organisms have life cycles that alternate between sexual and asexual reproduction.

Aphids reproduce both sexually and asexually. They have multiple generations where females asexually give birth to nymphs, each of which is a clone of the mother, and who, in turn, give birth to more nymphs (Figure 2.1.22).

This ability to rapidly increase in population to take advantage of environmental conditions is indicative of organisms grouped by yet another form of classification. This form of classification divides all organisms into two main groups: ***r*-strategists** and ***K*-strategists**.

FIGURE 2.1.22 Through both sexual and asexual forms of reproduction, aphids are able to take advantage of food sources with a rapid increase in population.

Organisms that are *r*-strategists generally (with some exceptions) have short lives, high (exponential) rates of reproduction, are quick to mature, have a strong sex drive and have little care for their offspring. They live in unstable habitats where conditions are often of a feast or famine nature. This results in ***r*-selection** where natural selection results in rapid appearance and removal of individuals, and quick dynamic changes in the population. Examples of these organisms are corals, insects, rodents and bacteria (Figure 2.1.23a).

K-strategists occupy stable environments and are generally much longer lived. Under ***K*-selection**, they produce fewer offspring but they expend a great deal of energy and time in nurturing their offspring, increasing their offspring's chances of reaching sexual maturity. *K*-strategist organisms generally take much longer to achieve sexual maturity and, being generally larger, have longer gestation periods. Examples of these organisms are elephants, sea turtles and fish such as the orange roughy (Figure 2.1.23b).

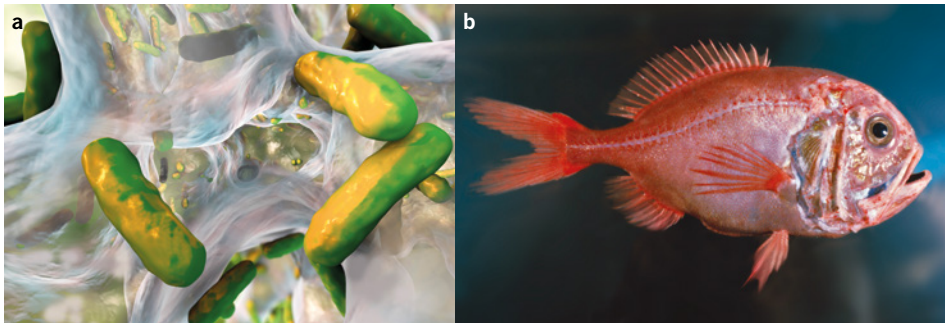


FIGURE 2.1.23 (a) The bacterium *Pseudomonas aeruginosa* is an *r*-strategist organism. (b) The orange roughy (*Haplostethus atlanticus*) is a *K*-strategist organism.

Classifying organisms by methods of species interaction

Organisms do not live in isolation. They interact with their **biotic** and **abiotic** environment. In other words, they interact both with each other and with their physical surroundings. Species interaction is used as a form of classification of organisms. By understanding these relationships, we can develop management plans for the conservation of the ecosystem in which these organisms live.

Organisms interact with one another in many ways. They eat one another (predation) and they also compete with one another for food, space, mates and nest sites (**competition**). **Symbiotic relationships** are also used to group organisms. With this understanding, special care can be taken to nurture these relationships in the community.

Even **disease** can be used as a classification grouping in ecosystems. A disease is any condition that impairs the normal activity of an organism. While diseases themselves can be grouped as **infectious** and **non-infectious**, it is within the infectious group that we have another means of classifying organisms that assists in identifying their impact upon an ecosystem or community. These groupings are pathogens, parasites, vectors and hosts.

Pathogens cause a disease in a host. While all pathogens are parasites, not all parasites are pathogens. Some parasites act as vectors (or transporters) for a pathogen to infect a host (Figure 2.1.24). Recognising which organisms fall into these categories and how they are assisted or impeded by overcrowding in populations, or favourable abiotic factors such as the presence or absence of aquatic systems, gives us data for the formulation of management plans for a particular ecosystem or region.

FIGURE 2.1.24 Some species of ticks (which are a parasite) act as a vector by carrying the bacterial pathogen *Borrelia burgdorferi*, which causes Lyme disease in humans.

Predation

As you will learn in Chapter 4, the energy flow through an ecosystem is a result of plants (producers) capturing solar energy and converting it to food for herbivores. The herbivores are in turn eaten by carnivores. There must be far greater numbers of herbivores than carnivores to support the predator population (Figure 2.1.25).

FIGURE 2.1.25 In any predator–prey relationship, there must be sufficient numbers of prey animals living in the environment to support a population of predators.

However, while prey numbers regulate predator numbers, the presence and type of predators directly impacts the structure and classification of an ecosystem. Predator–prey interactions affect the composition of species of prey and plant densities in a community, the behaviour and feeding habits of the prey animals, local biodiversity and the invasion of plant and animal species into an area.

An example of this is the regeneration of the ecosystems in Yellowstone National Park in Wyoming, USA, by the introduction of 14 grey wolves into the park (Figure 2.1.26). In the early 1900s, the US government initiated a wolf-eradication program in the park to preserve the populations of elk and protect the stock on cattle ranches bordering the park. By 1935, the grey wolf was eradicated and elk populations were left to increase relatively uninhibited by predation. Coyotes also benefited, as they were no longer being killed by wolves. Their increasing numbers decimated the population of smaller herbivores, such as rabbits and the smaller deer species.

The unchecked grazing by elk resulted in the removal of vegetation of watercourses, causing erosion and the breakdown in the structure and courses of the rivers and streams. The changed environment also affected the biodiversity of the park. Many insect, bird and small mammal species could not survive with the alteration of their habitats.

The controversial reintroduction of the grey wolf from Canada in the mid-1990s radically altered the feeding behaviour of the elk herds. Now the elk, as prey again, restrict themselves to the more heavily forested areas, leaving the vegetation to regenerate and re-establish along streams. The re-emergence of missing plant species has prompted the return of the animal species that had left the park, and watercourses have stabilised, creating an environment suitable for the return of aquatic plants and animals. The numbers of coyote were reduced, allowing the return of the smaller prey mammals and the subsequent reduction of undergrowth vegetation, which in turn enables the re-establishment of forest tree plant species.

FIGURE 2.1.26 The reintroduction of the grey wolf into Yellowstone National Park restored the complex biodiversity of the park.

Symbiosis in conservation

The kakapo (Figure 2.1.27a), a rare, flightless, nocturnal New Zealand parrot is a *K*-selected animal whose breeding cycles are closely linked to the fruiting cycles of its major food plants, in particular, a variety of podocarp tree known as rimu (Figure 2.1.27b). The rimu reproduction cycle stretches over three summers. In the first summer, the male and female gametes begin to develop, in the second summer the pollination cycle begins, and fertilisation occurs in the spring of the third season. This produces 'fruit', which ripens and causes the unprotected seeds to fall. The unripe seeds just prior to the third season are a major part of the diet of the adult kakapo. It is hypothesised that phytohormones in the unripe seeds stimulate egg production and assist in yolk formation in the female birds.

The 2017 census of the kakapo population stands at less than 160 birds. Kakapos are dependent on the rimu reproduction cycle and only breed when there is a significant production of seeds, which may not happen for three to five years. Kakapos breed only as a population, not as individuals, so five or more years may pass with no offspring being produced.

The rimu was heavily harvested for its timber during European settlement of New Zealand and it can take from 200 to 400 years for the rimu to attain maturity as a rainforest tree. Accordingly, New Zealand conservation reserves, such as Zealandia in Wellington, have a 500-year conservation plan as they replant key species to help regenerate populations of rare and endangered birds and reptiles on the main north and south islands of New Zealand. Conservation plans benefit from growing scientific knowledge, such as the classification and understanding of the symbiotic relationship between the kakapo parrot and the rimu tree.

FIGURE 2.1.27 The (a) kakapo is a *K*-strategist dependent on the (b) rimu, whose unripe seeds influence the reproductive hormones of the kakapo.

Phylogenetic classification

The Linnaean system of classification was based only on the morphological characteristics of organisms, especially their reproductive structures. It was created before scientists theorised how organisms evolved. Once the theory of evolution became accepted by scientists, biologists began to analyse possible evolutionary histories of organisms by comparing genetic sequences and molecular structures (phylogeny), and use this information to classify them. This is known as **phylogenetic classification**.

Patterns of morphological or genetic sequences (or both) can be represented in a branching diagram called a phylogenetic tree. The phylogenetic tree, which is like a family tree, is a useful way to show the evolutionary relationship between organisms, which is called a **phylogeny**. Molecular phylogeny is referred to as **cladistics**, that is, when similarities in molecular sequences are used to define a group of organisms (as in a group on the tree that contains an assumed common ancestor and its lineal descendants, also known as a clade).

For example, the sugar glider (*Petaurus breviceps*) and the yellow-bellied glider (*Petaurus australis*) belong to the same genus *Petaurus* and can be grouped together (Figure 2.1.28).

Leadbeater's possum (*Gymnobelideus leadbeateri*) does not glide, but it shares many features with the sugar glider and yellow-bellied glider, so together they form a larger group of possums (Figure 2.1.29).

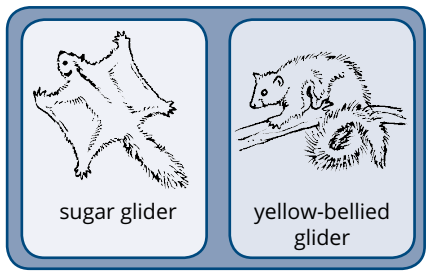


FIGURE 2.1.28 The sugar glider (*Petaurus breviceps*) and yellow-bellied glider (*Petaurus australis*) are morphologically similar and are placed into the same genus.

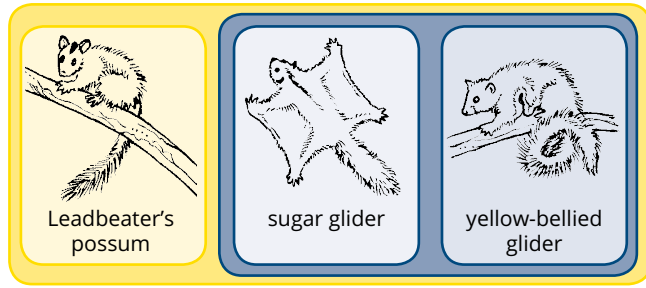


FIGURE 2.1.29 Leadbeater's possum (*Gymnobelideus leadbeateri*) does not glide, so it is not placed into the same genus as sugar gliders and yellow-bellied gliders.

The striped possum (*Dactylopsila trivirgata*) is part of an even larger group with these other animals. This pattern of groups can be represented by the Linnaean system (Figure 2.1.30) or a phylogenetic tree (Figure 2.1.31). A phylogenetic tree is like a family tree. Each branch point shows the most recent common ancestor of the organisms. From the phylogenetic tree, it can be deduced that the sugar glider is the closest relative of the yellow-bellied glider, and that the common striped possum is the most distant relative. The tree, and the Linnaean classification, depicts the evolutionary history of this group of possums.

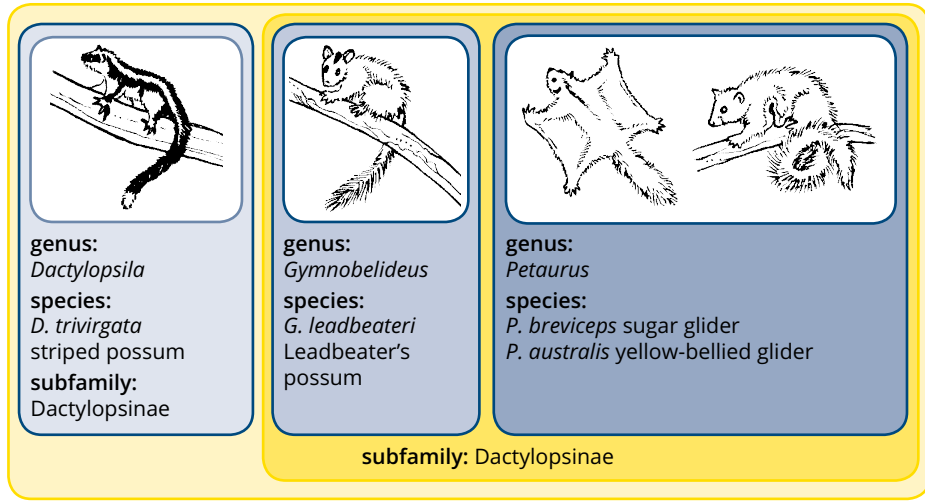


FIGURE 2.1.30 A classification of four possum species using the Linnaean system.

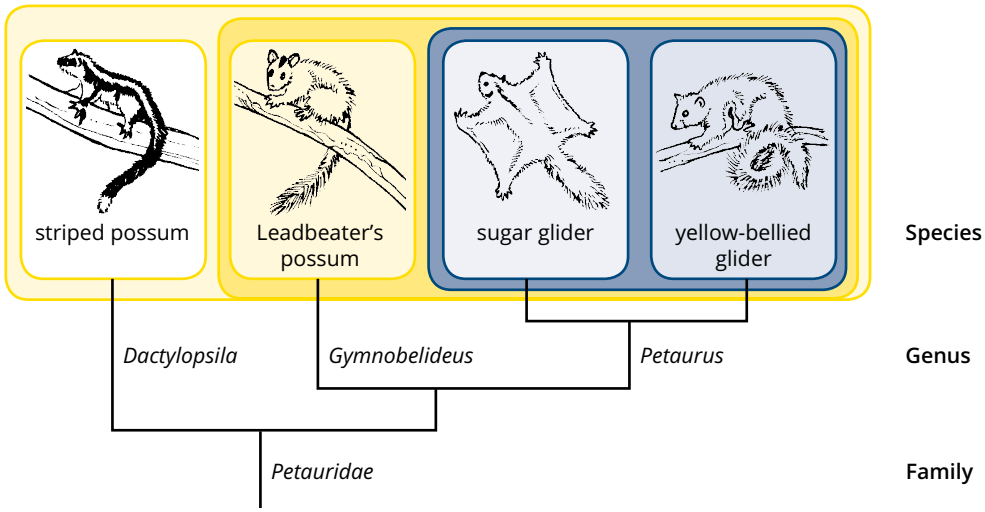


FIGURE 2.1.31 The phylogenetic tree shows that while the sugar glider is the closest relative of the yellow-bellied glider, the striped possum is a much closer relative to Leadbeater's possum than previously inferred.

When changes in mitochondrial DNA over time are considered, the phylogenetic tree can show previously undetected relationships between different genera and species. The theory and application of this analytical method will be explained in the next module.

CLASSIFYING ECOSYSTEMS

Every organism occupies an ecological niche, and the community of all living organisms interacting with one another and the physical abiotic factors around them forms a specific type of ecosystem. Understanding the interactions of individual organisms in different types of ecosystems is vital to managing and conserving organisms as a natural resource (for example, hardwood timber harvesting in old-growth forests or managing species catches in coral reef systems) or preserving ecosystems to prevent organisms from becoming extinct.

Ecosystems are composed of various habitats. These may be as small as a sweat gland pore inhabited by bacteria (**microhabitat**), or massive **ecoregions** covering large biogeographical areas. It is important to understand that the individual organisms, and the variety of habitats present, interact with, and are interdependent upon, one another through energy flows and nutrient cycles.

Reliable data is essential for efficient classification of ecosystems. Weather stations provide information of temperature, precipitation and humidity. Satellite imagery can map actual vegetation and landform types. On the ground, researchers gather data on both biotic and abiotic factors. The pooling of this information over time has increased our ability to predict the effects of climate change, deforestation, urbanisation and development, as well as allowing us to monitor the effects of our land management programs. The detailed mapping of ecosystems gives regional and national authorities the data required for the allocation of funding for habitat and species conservation, and for the development of efficient fire management schemes.

Australia's bioregion framework

The World Wide Fund for Nature has a mission to conserve nature and reduce the most pressing threats to the diversity of life on Earth. The Fund has used the division of the world into eight biogeographical regions based on landform types, climate and vegetation types:

- Nearctic (comprised mainly of North America)
- Palearctic (Eurasia and North Africa)
- Afrotropic (Sub-Saharan Africa)
- Indomalaya (South Asian subcontinent and South-East Asia)
- Australasia (Australia, New Zealand and other neighbouring islands)
- Neotropic (South America and the Caribbean)
- Oceania (Polynesia, Fiji and Micronesia)
- Antarctic (Antarctica).

As part of the Australian Government's National Reserve System, the Australian landmass has been divided into 89 **bioregions**, and 419 subregions (Figure 2.1.32). Bioregions are 'large, geographically distinct areas of land with common characteristics such as geology, landform patterns, climate, ecological features and plant and animal communities' (Australian Government).

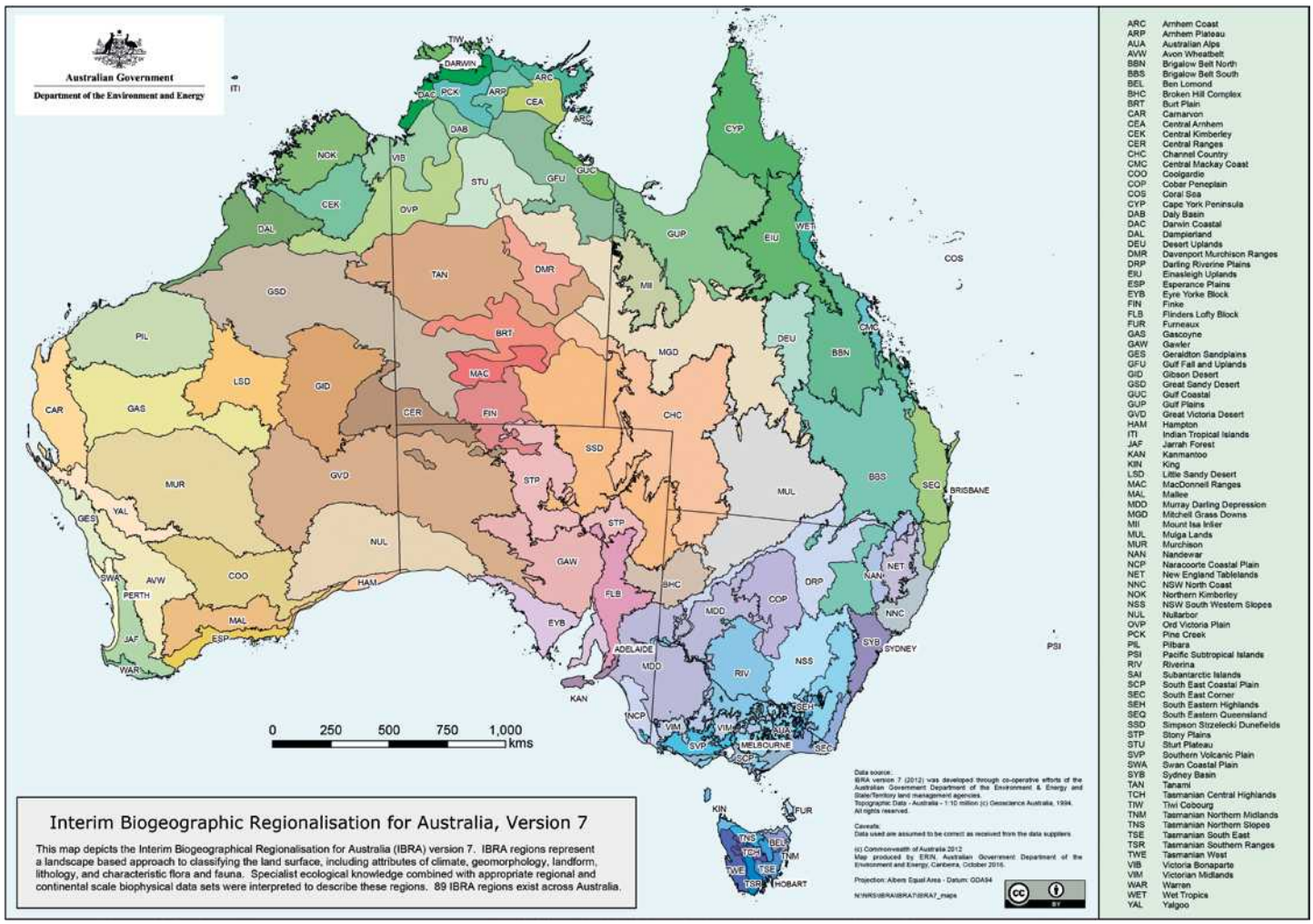


FIGURE 2.1.32 This map depicts the 89 bioregions of Australia.

The Queensland Government uses a three-step method to classify regional ecosystems. It is used to identify habitats, the conservation of endangered species, the regulation of mining and land clearing and also fire management. The three-step method is detailed in *Queensland's Regional Ecosystem: Building and maintaining a biodiversity inventory, planning framework and information system for Queensland*, published by the Queensland Herbarium and the Department of Science, Information and Technology.

The three steps are:

1 identification of the 13 bioregions that occur in Queensland (Figure 2.1.33)
2 identification of which of the 12 types of landforms the ecosystem is on (Table 2.1.1 on page 20)
3 identification of the 185 vegetation communities using Webb's classification for rainforest and a modified Specht classification system (Table 2.1.2 on page 23) for non-rainforest vegetation.

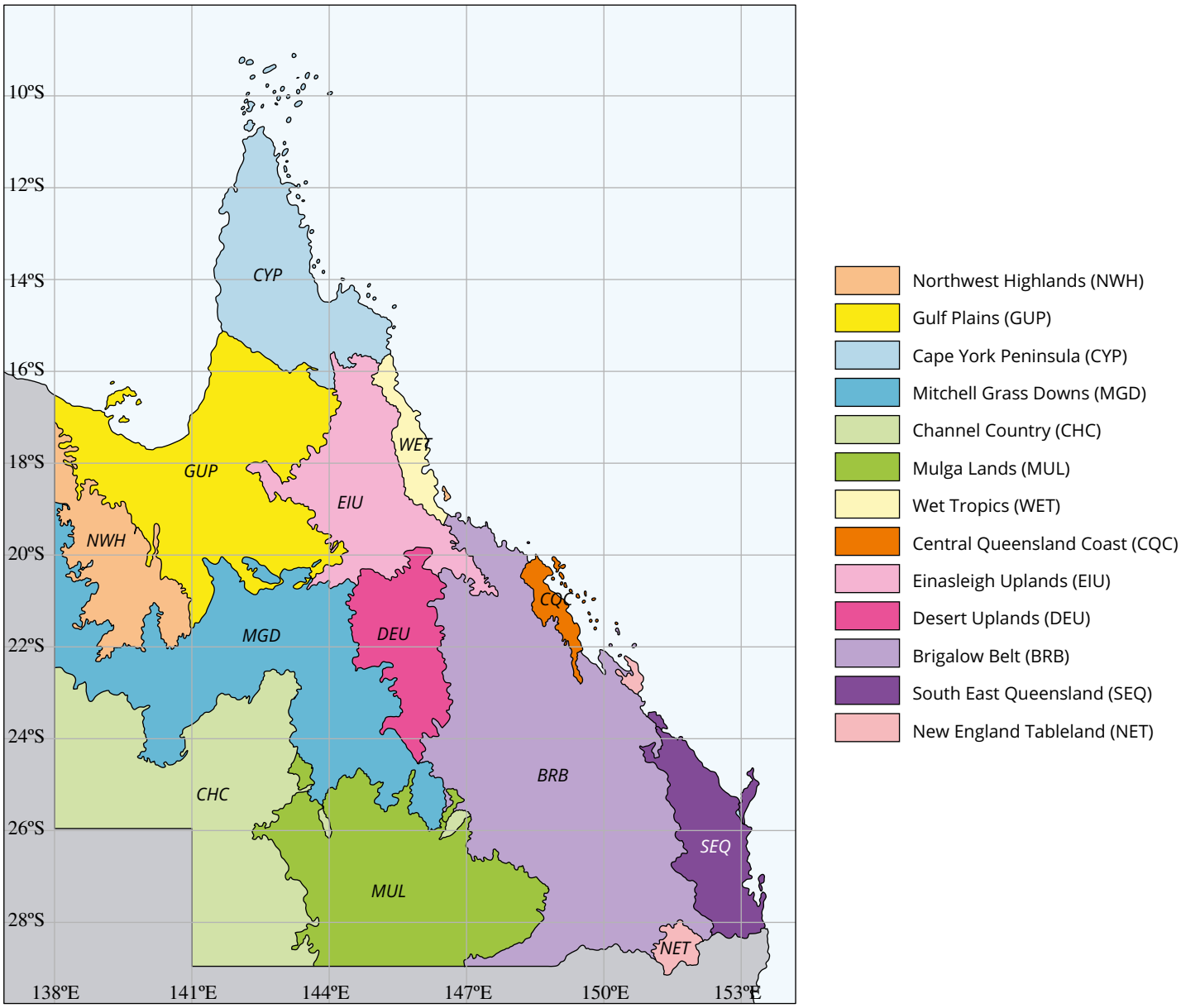
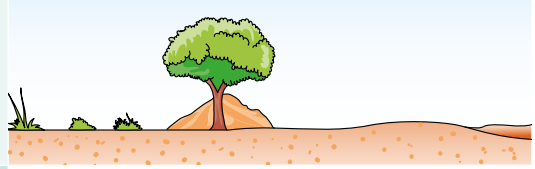
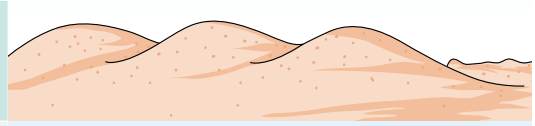
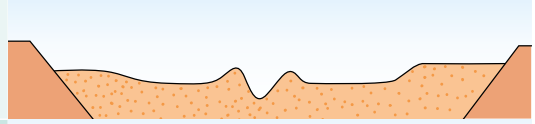
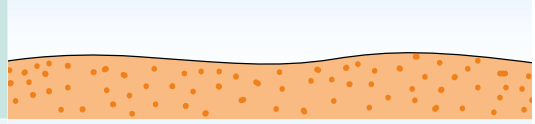
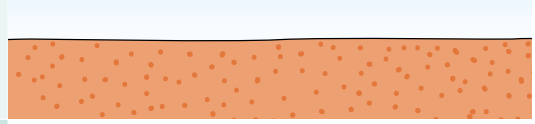
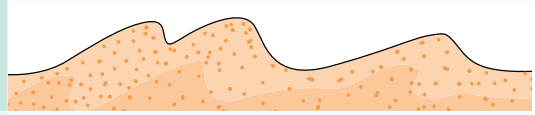
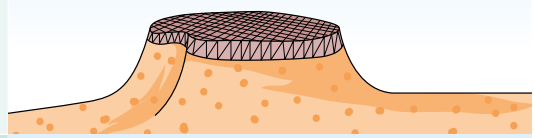
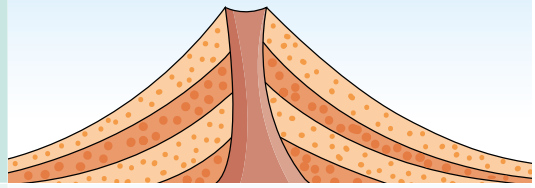
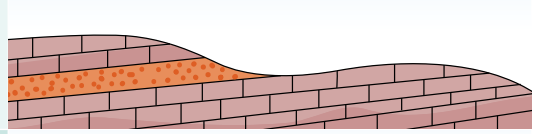
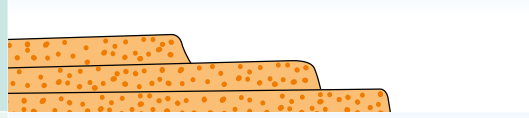
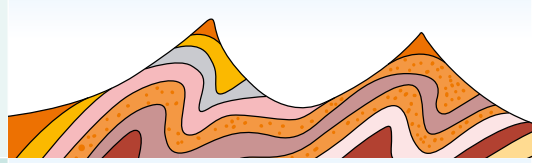
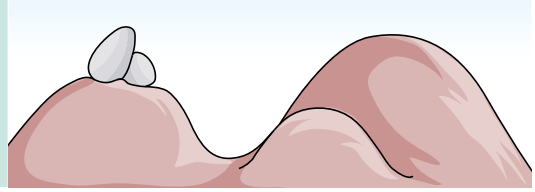


FIGURE 2.1.33 Bioregions of Queensland.

TABLE 2.1.1 Land zones of Queensland

Land zone	Description	Image
1	tidal flats and beaches	
2	coastal dunes	
3	alluvial rivers and creek flats	
4	clay plains	
5	old loamy and sandy plains	
6	inland dunefields	
7	ironstone jump-ups	
8	basalt hills and plains	
9	undulating country on fine grained sedimentary rocks	
10	sandstone ranges	
11	hills and lowlands on metamorphic rocks	
12	hills and lowlands on granitic rocks	

For example, a regional classification of Daintree National Park (Figure 2.1.34) would be 7.12.48, which means bioregion 7 (wet tropics), land zone 12 (hills and lowlands on granitic rocks) and vegetation community 48 (wind-sheared notophyll vine forest on exposed granite and rhyolite ridge crests and steep slopes). This type of identification is now possible online using the Queensland Government's 'Search regional ecosystem descriptions' website.

FIGURE 2.1.34 Daintree National Park (RE 7.12.48) is a regional ecosystem (RE) composed of a rainforest vine community on steep-sloped granite hills in the wet tropics.

Ecosystem classification types

There are four other major types of ecosystem classification systems that are of interest to scientists worldwide:

- Holdridge life zone classification scheme
- Specht's classification system
- Australian National Aquatic Ecosystem (ANAE) classification system
- European Nature Information System (EUNIS) habitat classification system.

Holdridge life zone classification scheme

The Holdridge life zone system classifies life zones according to average annual rainfall (mm), average biotemperature (the mean of all temperatures between 30°C and 0°C, and all temperatures above and below this range adjusted to 0°C) and altitude (m). These parameters can be measured across many regions and ecosystems across the globe, including Australia. It also considers the latitude of a region, humidity and potential evapotranspiration rate (Figure 2.1.35 on page 22). The life zones can be further subdivided into associations based on more detailed climate data, soil conditions and topography. It does not specify homogenous communities, and there may be several vegetation types within a life zone based on the soils and the presence or absence of open water. This method of classification has largely been superseded in Queensland by the Regional Ecosystem classification.

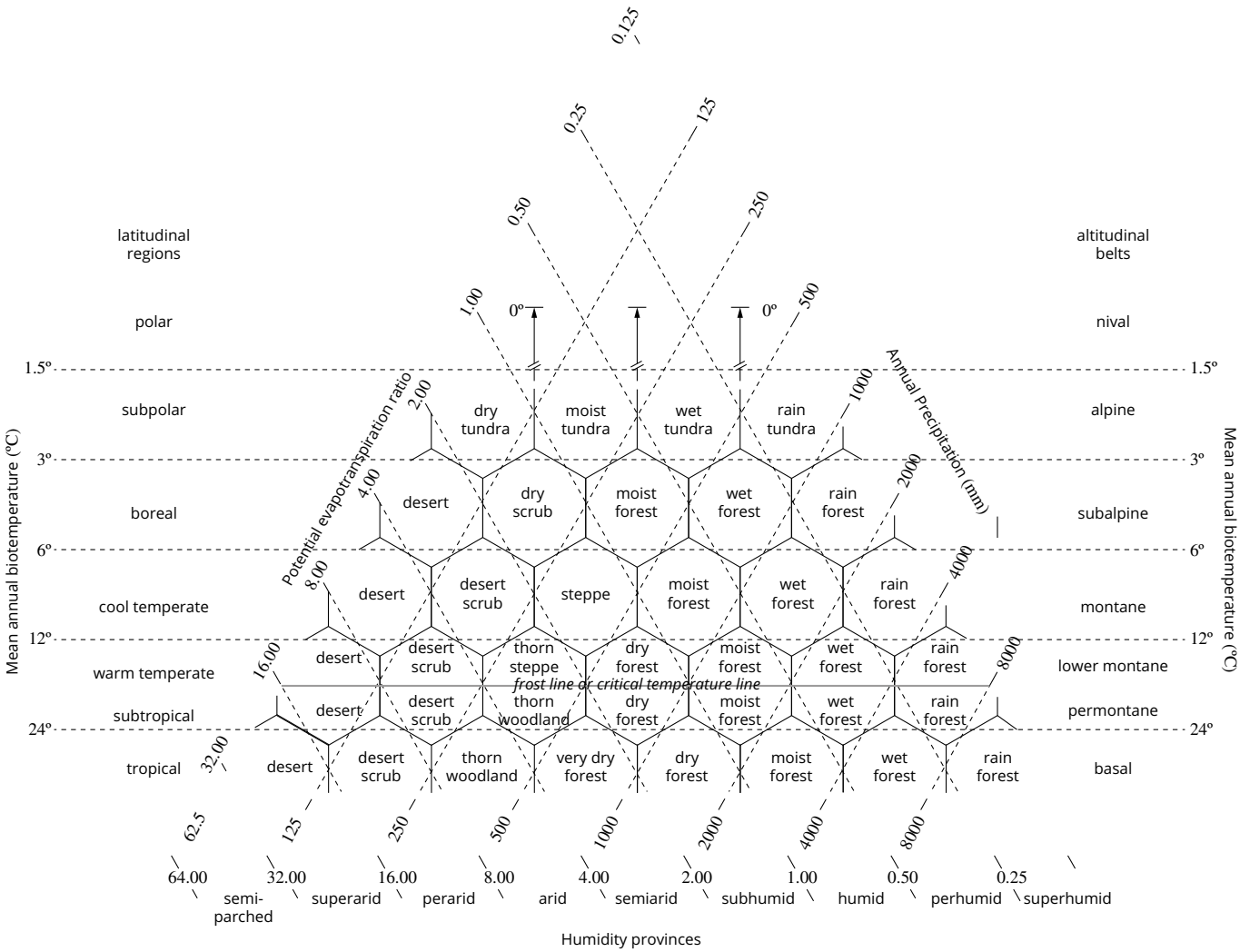


FIGURE 2.1.35 Chart of Holdridge life zone system classifications.

Specht's classification system

Developed by RL Specht in the 1970s, this system classified ecosystems by their dominant types of vegetation and canopy cover. The type of plant is that which forms the tallest or dominant layer in a community. The tallest layer can be a tree (defined as a woody plant more than 5 m tall and having a single stem), a shrub (a multi-stemmed woody plant), or a grass or herb. The density, or cover of the canopy, is estimated according to the amount of sunlight blocked out: 70–100%, 30–70%, or less than 30%.

Using these two estimates, a plant community in Australia can be classified as shown in Table 2.1.2. This classification system is still used as a modified version for regional ecosystems in Queensland. The vegetation is described by the stratum that contributes the most above-ground biomass. The tallest strata (if it is not the predominant strata) are called the emergent layer. The height and canopy cover of each layer is measured independently of the other.

TABLE 2.1.2 Classifying Australian plant communities

Dominance	Canopy cover		
	70–100%	30–70%	<30%
Trees dominant	**Closed-forest** Little sunlight penetrates to the floor of the forest. 'Closed-forest' is a general term that includes the tropical, warm temperate and cool temperate rainforests scattered down the eastern edge of Australia. Many mangrove forests in tropical Australia are also classed as closed-forests.	**Open-forest** This is usually dominated by eucalypt species. They can be further subdivided into tall open-forest (trees above 30 m), open-forests (trees 10–30 m) and low open-forests (trees 5–10 m). The differences in tree height relate to the amount of rainfall for the area.	**Woodland** This occurs in drier areas and has trees spaced further apart than in a forest. More light reaches the lower layers in a woodland than in a forest. Australian woodlands are dominated by *Eucalyptus*, cypress pines (*Callitris*), she-oaks (*Casuarina* and *Allocasuarina*), paperbarks (*Melaleuca*) and wattles (*Acacia*).
Shrubs dominant	**Scrub** This is a dense plant community dominated by shrubs 2–8 m tall. Mallee and coastal tea-tree vegetation are two examples of scrub communities.	**Open-scrub** This is less dense than scrub.	**Shrubland** Shrubland such as mulga (dominated by *Acacia aneura*) is sparse. **Heathland** This is also dominated by shrubs, but its tallest plants are less than 2 m high.
Grasses and herbs dominant	**Herbfields** This is dominated by a variety of non-woody plants other than grasses. They are important communities in the alpine regions of south-eastern Australia, where they include plants such as snow daisies. Grasslands are dominated by grasses. Large hummocks of porcupine grass (*Triodia*) form a peculiarly Australian plant community in the driest areas of Australia.		

ANAE classification system

The Australian National Aquatic Ecosystem (ANAE) was designed as a flexible and consistent three-level approach to classifying aquatic ecosystems (Figure 2.1.36).

Level 1 uses broad-scale regional classifications from existing datasets sets such as Interim Biogeographic Regionalisation for Australia, Australian Soil Resources Information System (ASRIS), Integrated Marine and Coastal Regionalisation of Australia and Australian Hydrological Geospatial Fabric (Geofabric) developed by the Bureau of Meteorology as well as climate classifications based on Köppen's classification of six world climates.

Level 2 uses a finer scale for each of the above data sources, which provide increased contextual information for each of the categories. For example, the six Köppen climate classifications are refined to 27 subcategories in Australia.

Level 3 focuses on two water-dependent classes of the landscape—surface waters and subterranean waters/aquifers. These classes are then further broken down into systems. Each system is then examined for attributes such as water type, vegetation, substrate, water source and porosity to determine a habitat classification.

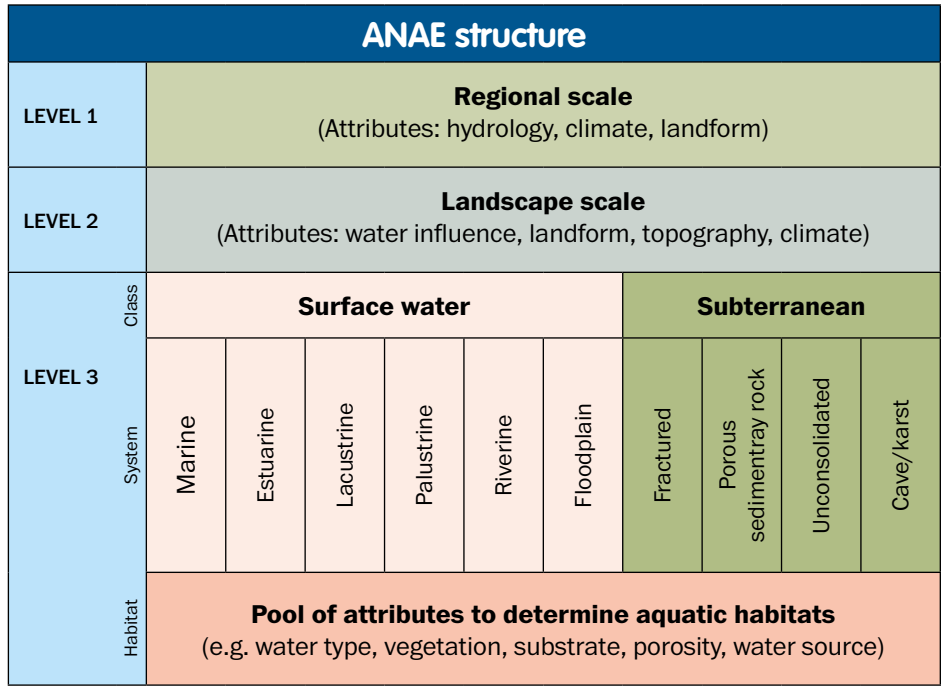


FIGURE 2.1.36 Structure and levels of the Australian National Aquatic Ecosystems classification framework.

EUNIS habitat classification system

The European Nature Information System (EUNIS) habitat is a pan-European system and covers all types of aquatic, terrestrial and benthic marine habitats that may be either natural or artificial. The system gives members of the public access to European data from different databases and organisations by means of three interlinked modules covering sites, species and habitat types.

Essentially, EUNIS is an interactive, criteria-based key that uses sequential questions with a choice of answers. The user progresses through the key until they have a definitive classification for their habitat, species or site.

WS 3.1.4

2.1 Review

SUMMARY

- Life on Earth consists of a great diversity of living organisms, which we call biodiversity.
- Classification is the grouping of organisms on the basis of features that they have in common.
- Taxonomy is the naming of these groups scientifically.
- Classifying and naming organisms enables:
  - effective communication between scientists around the world
  - grouping of organisms in a hierarchy
  - quick and efficient identification of living things
  - scientists to learn about the evolutionary relationships between different organisms
  - recognition of an organism that is beneficial, poisonous or dangerous.
- The scientific name of each species consists of two parts: genus and species. This two-part naming system is called the binomial system.
- The genus name always begins with a capital letter, and the species name begins with a lower-case letter. The names are italicised when typed and underlined when handwritten.
- The species is the basic unit of classification. Members of a single species are so similar to each other that they can breed and produce fertile offspring that resemble one of the parents.
- Classification of organisms is based on morphological or genetic characteristics, or both.
- Biological classification is hierarchical. Species are classified together into genera, genera into families, families into orders, orders into classes, classes into phyla and phyla into kingdoms.
- Characteristics of species can be used to construct a phylogenetic tree to show the evolutionary relationships between organisms.
- Genetic information from DNA analysis sometimes causes species to be reclassified into a different group.
- The most widely used classification includes the five kingdoms: Protista, Plantae, Fungi, Animalia and Plantae.
- Some classifications group organisms into three domains: Bacteria, Archaea and Eukarya. Eukarya includes the four eukaryote kingdoms: Protista, Fungi, Plantae and Animalia.
- There is a separate classification system for viruses, based on DNA, size, capsid structure and host organism.
- Classification can also be based on reproductive strategies: *K*-strategists have fewer offspring and expend a great deal of energy nurturing them, while *r*-strategists have large numbers of offspring but take little or no care of them.
- Ecosystem classification is essential for managing and conserving living natural resources and their habitats to prevent extinction of vulnerable species and to develop effective management programs.
- Regional ecosystem classification in Queensland is based on the bioregion, land zone and vegetation community.
- Other common classification systems include the Holdridge life zone classification scheme, Specht's classification system, ANAE and EUNIS.

KEY QUESTIONS

Retrieval

1 List four benefits of classifying and naming organisms.

2 Recall three definitions of species.

3 List the groups representing the different levels of classification in the Linnaean system from lowest to highest.

4 Define ecosystem.

Comprehension

5 Describe what an interspecific hybrid is and explain why they are not classed as a separate species. Use an example in your response.

6 Explain the binomial system of scientifically naming organisms and use an example in your explanation.

7 Discuss why Holdridge's chart, developed in Central America, could be used to classify Australian ecosystems.

8 Compare the benefits of *K*-selected and *r*-selected species.

continued over page

2.1 Review *continued*

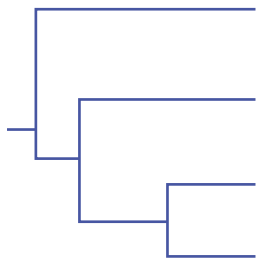
Analysis

9 A field biologist is in the process of classifying plant communities that will be in the path of a proposed railway from the Central Highlands of Queensland to a new port on the North Queensland coast. For one particular patch of bush she makes the following notes in her logbook: 'Main vegetation *Eucalyptus* trees 15–20 m in height. Average transects canopy cover 45%.' Use Table 2.1.2 (page 23) to classify the plant community that she is in.

10 The following table compares the group names for red fox, Arctic fox, dog and cat. Organise the names into the phylogenetic tree below.

Group names of selected species

Group	Red fox	Arctic fox	Dog	Cat
Kingdom	*Animalia*	*Animalia*	*Animalia*	*Animalia*
Phylum	*Chordata*	*Chordata*	*Chordata*	*Chordata*
Class	*Mammalia*	*Mammalia*	*Mammalia*	*Mammalia*
Order	*Carnivora*	*Carnivora*	*Carnivora*	*Carnivora*
Family	*Canidae*	*Canidae*	*Canidae*	*Felidae*
Genus	*Vulpes*	*Vulpes*	*Canis*	*Felis*
Species	*Vulpes vulpes*	*Vulpes lagopus*	*Canis lupus*	*Felis catus*

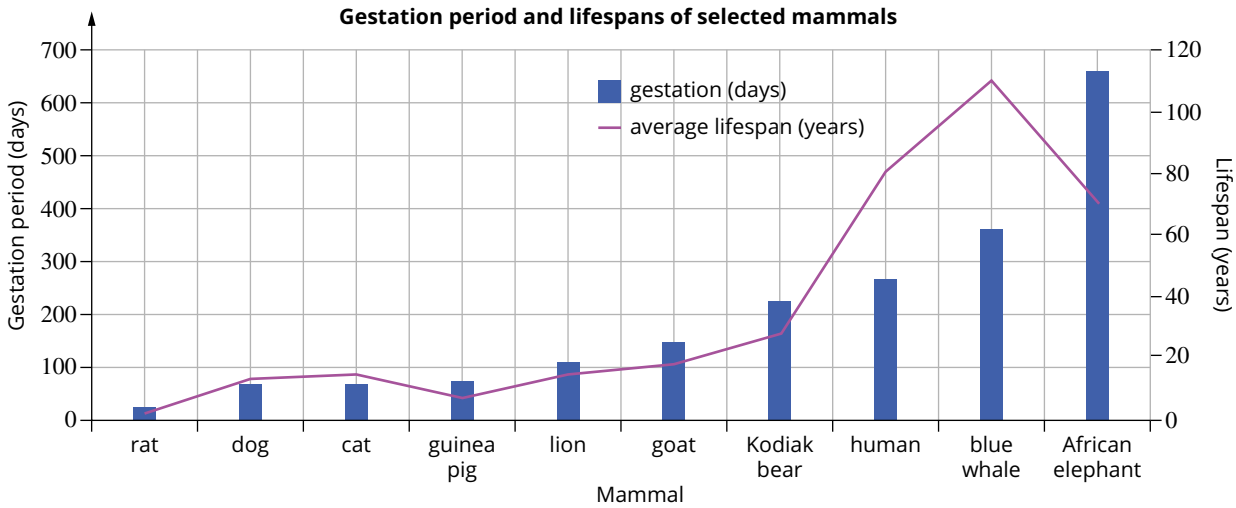


11 Use the graph below showing the gestation periods and lifespans of various mammals to:

- **a** determine the gestation period and life span of rats, guinea pigs, lions and whales
- **b** infer whether the selected species are *K*-selected or *r*-selected.

12 Identify the correct characteristics for classifying an ecosystem.

- **a** based on geo-referenced qualitative data, as the more data-based a system is the more accurate and less subjective it will be in classifying an ecosystem
- **b** requires subjectivity in applying any classification rules that the system uses
- **c** reflects as closely as possible the forces driving the ecosystem, as ecosystems are driven primarily by climatic factors, followed by soil types, landform features and biotic factors
- **d** be hierarchal and recognise and evaluate the varying levels of complexity that will occur in the chemical, biological and ecological facets of the ecosystem
- **e** adaptable for expanding or contracting complexity scales by having classification units that are fixed and cannot be combined or separated as the complexity of the ecosystem requires (for example, by combining the features of 'Tropical' and 'Rainforest')
- **f** applicable to the entire world and must not be able to account for changes in ecosystems due to latitude
- **g** demonstrably able to be validated with independent ecological data



2.2 Processes of classification

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- define the term clade
- recall that common assumptions of cladistics include a common ancestry, bifurcation and physical change
- interpret cladograms to infer the evolutionary relatedness between groups of organisms
- analyse data from molecular sequences to infer species evolutionary relatedness.

Before molecular techniques were available, structural (or morphological) and functional similarities were the main evidence used for classification. Humans, chimpanzees, gorillas and orangutans each have forelimbs with hands and five digits (fingers). These morphological similarities can be used to classify these organisms into similar groups and as evidence to support the theory that they all descended from a common ancestor.

However, anatomical similarities may also be due to convergent evolution of organisms that did not share a recent common ancestor and have very different DNA sequences.

In this module, you will learn how amino acid and DNA sequences are used for classification and as evidence of relatedness between species, how comparison of mitochondrial DNA sequences can be used to indicate the existence of a shared common ancestor, and how the DNA hybridisation technique is used to compare DNA molecules of different species.

DETERMINING AND INFERRING RELATEDNESS BETWEEN SPECIES

All living organisms on Earth are inferred to have once shared a common ancestor. If two species have a very similar set of proteins, chromosomes or DNA sequences, it is used as molecular evidence that they shared a recent common ancestor. 'Recent', in evolutionary terms, may be hundreds of thousands, even millions, of years. Interpreting molecular similarities is used in classification and can group organisms into clades of various similarities.

If two populations become isolated from each other, they will accumulate differences in their DNA sequences over time. Changes in nucleotide sequences are caused by mutations, which may be passed on during reproduction if it occurs in the germ line cells (gametes) (Chapters 5 and 7 cover this in more detail). As time passes, more mutations occur within the two isolated populations and the sequence of nucleotides in their DNA becomes more different, gradually causing divergence (Figure 2.2.1). Eventually the genetic make-up of the two isolated populations will be so different that they become two separate species.

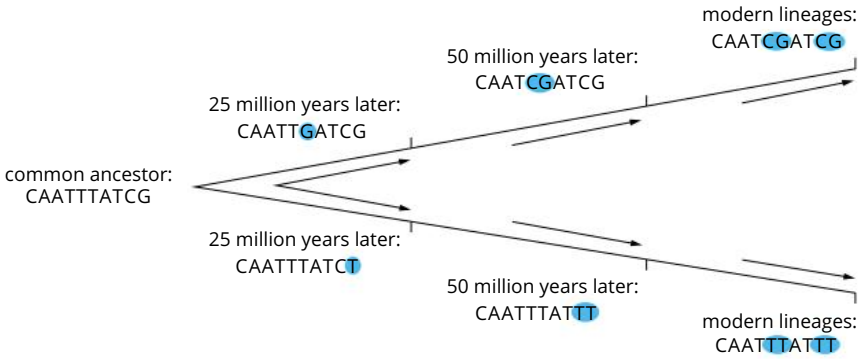


FIGURE 2.2.1 When species diverge, they accumulate different mutations in their nucleotide sequences. The more time that passes, the more mutations they accumulate.

A molecular clock is a technique that uses the rate of accumulation of mutations in DNA to calculate how long ago organisms diverged from one another. This hypothesis states that changes in DNA and proteins are constant over evolutionary time and across different lineages. However, the rate of genetic change is not constant and, as we only know about changes that can be observed today, a molecular clock will underestimate the divergence that has occurred.

The greater the difference in the DNA sequences between two species, the more time is inferred to have passed since they shared a common ancestor. For example, there are more differences between the DNA sequence coding for the cartilage protein of a frog and a dog than between a frog and a toad. This is interpreted as evidence that a dog and a frog have a more distant common ancestor than a frog and a toad and have therefore had more time to accumulate genetic changes.

Mutations occur regularly as a species evolves—like 'molecular clockwork'. As time passes, mutations accumulate in DNA, resulting in genetic differentiation and divergence of species.

Differences in amino acid sequences

Different proteins are hypothesised to mutate at different rates. Figure 2.2.2 shows the rate of genetic divergence, or **mutation rate**, of four different proteins over millions of years. The proteins with the slower mutation rates, like histone IV, are highly conserved, indicating that they are involved in functions that are essential to the survival of the organism.

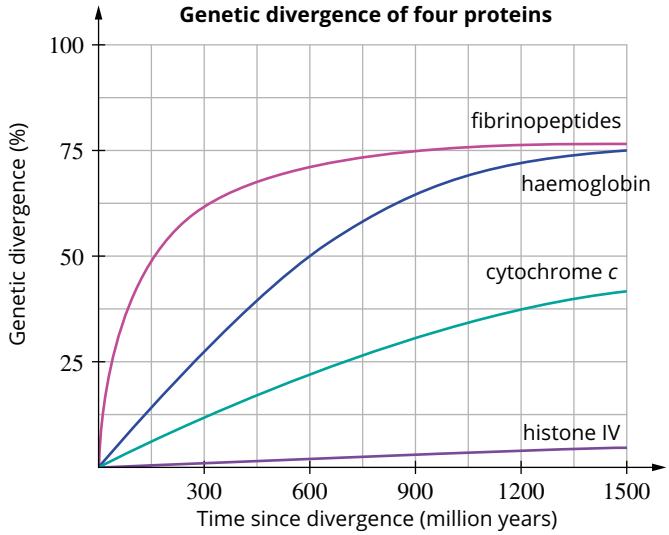


FIGURE 2.2.2 The genetic divergence of fibrinopeptides, haemoglobin, cytochrome *c* and histone IV.

As species diverge, differences in the amino acid sequences of their proteins increase due to the accumulation of mutations. Organisms that share a more recent common ancestor have fewer differences in their amino acid sequences. Figure 2.2.3 shows that, for one particular amino acid sequence, humans and rats have fewer differences than humans and zebrafish.

```
                421       431       441       451           461       471       481
                  |         |         |         |             |         |         |
H. sapiens      sdseeegeggrknssnfkkakrvktedekekdpeekke----vteeektkeekpeakgvkeevkla
R. norvegicus   sdsdeegeggrknssnfkkakrvktedekekdpeekke----vteeektkeekpeakgvkeevkma
D. rerio        sdsedegqggrrnaanykkpkrvkteee--kdgeekkgeptdvkeeekaseekmdtkgpkeelktv
```

FIGURE 2.2.3 Amino acid alignment of part of the histone deacetylase 1 protein. Conservative amino acid substitutions do not cause a change in the protein. Semi-conservative amino acid substitutions result in an amino acid being replaced by one that is similar in structure but has different biochemical properties. This may lead to a change in the protein. Non-conservative mutations result in an amino acid being replaced by one that is very different, and this usually leads to major changes in the protein and its function. The organisms that share a more recent common ancestor have fewer differences in their amino acid sequences (e.g. *Homo sapiens* (human) and *Rattus norvegicus* (rat) compared to *H. sapiens* and *Danio rerio* (zebrafish)).

The number of different amino acids in the same protein between species can be analysed to group together organisms in classification and suggest their relatedness. Table 2.2.1 shows the number of amino acid differences in the cytochrome *c* molecule between humans and selected organisms, thereby comparing the relatedness of humans to each of these species. For example, there are 51 amino acid differences in the protein sequence of cytochrome *c* between yeast and humans. This indicates a low level of relatedness. Cytochrome *c* is an important part of the electron transport chain in cellular respiration and is essential to an organism's survival.

TABLE 2.2.1 The number of amino acid differences in the cytochrome *c* molecule of different organisms

	Human	Rhesus monkey	Horse	Donkey	Sheep	Dog	Yeast
human	0						
rhesus monkey	1	0					
horse	12	11	0				
donkey	11	10	1	0			
sheep	10	9	3	2	0		
dog	11	10	6	5	3	0	
yeast	51	51	51	50	50	49	0

Genetic material used in classification is not just found in the nucleus of a cell. Mitochondria present in eukaryotic cells have their own genome, known as **mitochondrial DNA (mtDNA)**. In humans, mtDNA contains 37 genes that code for 2 ribosomal RNAs, 22 transfer RNAs and 13 proteins. Mitochondrial DNA has shown to be a highly reliable molecule for comparison and classification within related organisms. This is because it is unique, in that it is passed through the maternal line of sexually reproducing organisms. Offspring inherit mtDNA from their mother only (Figure 2.2.4), while nuclear DNA is passed on from both parents.

Mutations in mtDNA accumulate over time just as they do in nuclear DNA. However, because mtDNA does not have the same repair mechanisms as nuclear DNA, the rate of mutation in mtDNA is usually higher than in nuclear DNA (there are also some highly conserved regions). An added advantage of using mtDNA is that it is easier to obtain high yields of DNA, because most cells contain many mitochondria.

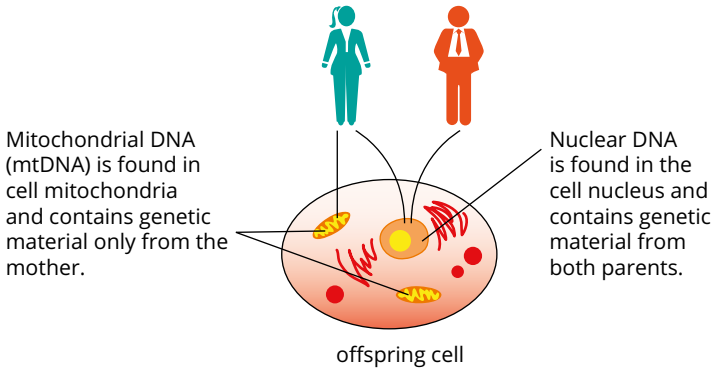


FIGURE 2.2.4 When a sperm and egg fuse, the sperm contributes nuclear DNA only. The egg contributes nuclear DNA as well as the mtDNA of the mitochondria in its cytoplasm. Therefore, mtDNA is only inherited through the maternal line.

Techniques for measuring genetic differences between organisms

The collection of DNA at a crime scene is a modern forensic tool that can be used to identify specific individuals who were at that crime scene. But genetic material is also used in establishing relationships between different species, not just of the same genus, but in entirely different kingdoms, or even domains. There are two major techniques used today: DNA hybridisation and the sequencing of DNA nucleotides.

DNA hybridisation

DNA hybridisation is a technique that can be used to determine the level of similarity between sections of DNA of two species. When a small section of double-stranded DNA is heated gently, the hydrogen bonds between complementary bases break and the two strands separate. As the two strands are cooled, the complementary bases match up again and the DNA becomes double-stranded once more. If DNA from two different organisms is gently heated to obtain single strands, mixed and then cooled, sections with similar nucleotide sequences will form hybrid double-stranded DNA, containing one strand from each organism (Figure 2.2.5).

For example, the following process would be followed to compare the region of DNA that produces the protein casein in humans and cows:

1. The casein gene is isolated from the nuclear DNA of each species using **gene probes** (small complementary sequences with an attached dye).
2. Both the human and cow double-stranded DNA are heated to 95°C to break the hydrogen bonds between complementary bases and separate the individual strands of DNA.
3. The individual strands of DNA of the two species are mixed together and allowed to cool. Where the nucleotide bases of the human and cow genes are complementary, hydrogen bonds will form, creating a strand of hybridised DNA.
4. The level of similarity is measured by reheating the hybrid DNA molecule. The temperature needed to separate half of these molecules is recorded as the melting temperature or thermal stability.

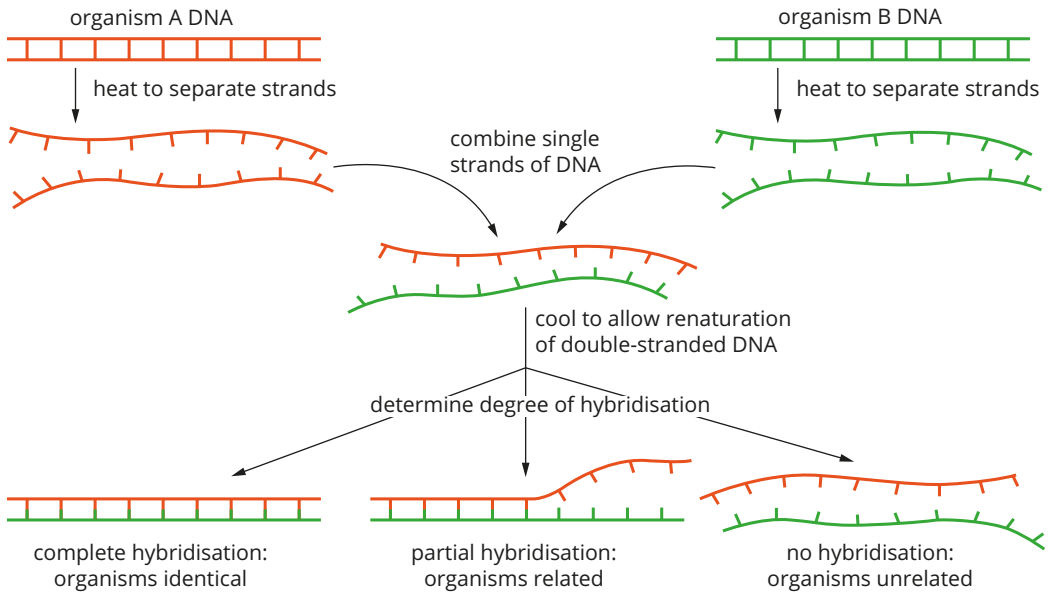


FIGURE 2.2.5 The steps involved in DNA hybridisation to determine the level of similarity. When the hybridised DNA strands are reheated, the strands that are completely hybridised will require a higher temperature to separate than the partially hybridised strands.

For any two species, the more complementary pairs the two strands have in common, the more hydrogen bonds will form and the more strongly the two strands of DNA will be bound together. This means that more heat will be needed to separate them. Therefore:

- high separation temperature indicates there are relatively more complementary nucleotides between the genes of two species
- a low separation temperature indicates there are fewer complementary nucleotides between the genes of two species.

Uncertainties and limitations of this technology arise from the assumptions that are made, and also the controls implemented, such as temperature and concentration of salt solution. It is assumed that when the individual strands of the two species are mixed together after heating, the likelihood of the DNA re-forming into double-stranded DNA with its own species or with the other species is equivalent. DNA hybridisation also assumes that damage to the DNA molecule during the process is negligible. As DNA strand bonding is thought to depend on random collision theory, the likelihood of either 75%, 50% or 25% of the DNA re-forming to form hybridised strands is equivalent. This will be randomly different from trial to trial, and it is unknown how much of the final mixture is hybridised. The final melting temperature or thermal stability result of this technique is interpreted on the assumption that all DNA hydrogen bonds formed are hybridised without DNA damage.

DNA sequencing

DNA hybridisation only provides an overall estimated measure of the degree of difference in the DNA sequences of different species for isolated genetic material. However, modern DNA sequencing techniques provide a more accurate measure of sequence differentiation by allowing a direct comparison of the nucleotide sequences. The exact number of nucleotide differences between species can then be determined (Figure 2.2.6). The details of some DNA sequencing technologies, how they work, their results and limitations are explained in Chapter 8.

a

	1	2	3	4	5	6	7	8	9	10	11
eucalypt (*Corymbia*)	C	C	C	–	T	C	T	T	T	T	T
mung bean (*Vigna*)	C	T	C	T	T	T	T	T	T	C	A
soybean (*Glycine*)	C	T	C	T	T	T	T	T	A	C	G
pine tree (*Pinus*)	C	C	T	–	C	C	C	C	C	C	C

b

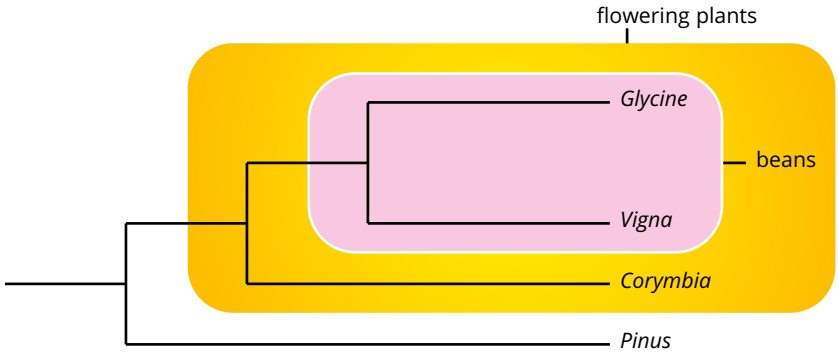


FIGURE 2.2.6 (a) Alignment of part of the sequence of the gene coding for ribosomal RNA of three flowering plants (a eucalypt, *Corymbia*, and two beans, *Vigna* and *Glycine*) and a pine tree (*Pinus*). It shows that the three flowering plants have bases in common (highlighted blue). The two beans have even more bases in common (highlighted green), indicating their high similarity and close relationship. (b) These DNA sequence differences and their evolutionary relationships are represented in a phylogenetic tree.

PHYLOGENETIC TREES

Phylogenetic trees (or phylogenies, also known as evolutionary trees) are branching diagrams that depict hypothesised evolutionary relationships by classifying organisms into groups. They are constructed using homologous features, both morphological and molecular, to infer the branching history of common ancestry between groups of organisms. As information regarding the evolutionary history of an organism is mostly unknown, scientists use evidence from the morphology and DNA or RNA sequences of living species, discerning commonality and differences, to reconstruct an inference of their evolutionary past. Such scientific investigations produce a number of possible phylogenetic trees as a result, then choosing one as the evolutionary hypothesis.

Today, most phylogenetic trees are built using DNA or RNA sequence data, and organisms are grouped on the basis of the similarity of their nucleotide sequences. Phylogenetic trees based on molecular characters (DNA or RNA nucleotides) can be used to compare any organisms, even if they seem to have very few characteristics in common (Figure 2.2.7). This is because the molecular characters on which the phylogenetic tree is based are the same for all organisms. However, the different mutations of different DNA or RNA regions need to be considered when constructing phylogenies. For this reason, phylogenetic trees are usually constructed using sequence alignments of the same gene in different organisms.

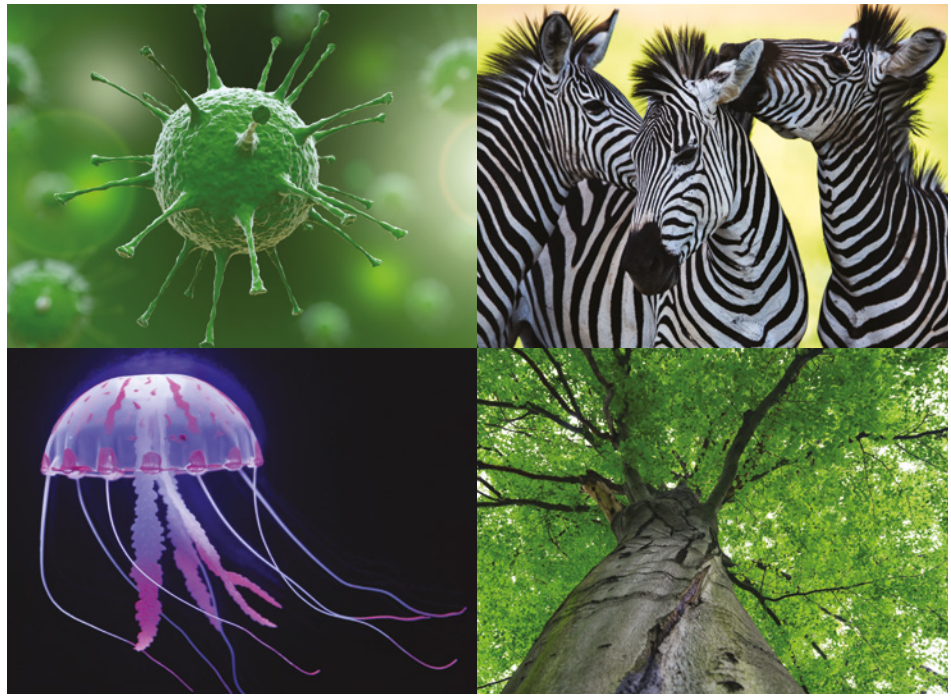


FIGURE 2.2.7 Phylogenetic trees based on molecular characters can be used to compare any organisms, even if they seem to have very few characteristics in common.

As you learnt in the previous section, comparison of DNA sequences is now possible, allowing the discovery and inference of phylogenetic relationships that were previously unknown and the reclassification of organisms into more accurate taxonomic groups. Molecular data, combined with morphological and ecological information, continues to develop our understanding of evolution and refine the system of biological classification (Figure 2.2.8).

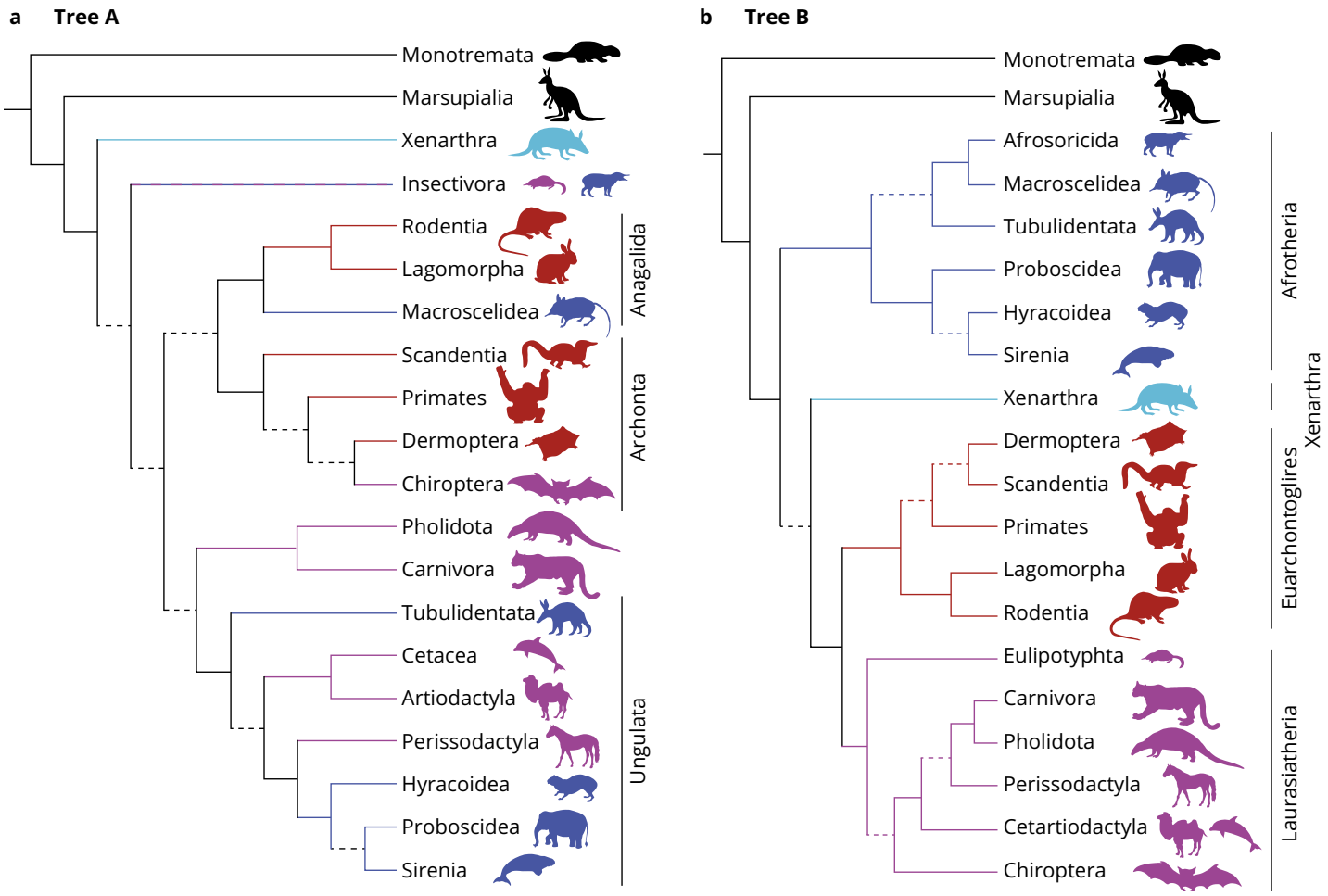


FIGURE 2.2.8 Two phylogenetic trees representing the evolutionary relationships of placental mammals: (a) Tree A was constructed using morphological data and (b) Tree B was constructed using molecular data. The trees are similar in appearance but the positioning of some taxa differs. The colours represent the four major groups of placental mammals. Marsupials and monotremes are included as a comparison (outgroup).

Building phylogenetic trees

Phylogenetic trees are diagrams that show evolutionary relationships between different groups of organisms. The groups compared in a phylogenetic tree can be different species, genera, phyla or any other level of Linnaean classification.

A phylogenetic tree is built by placing taxa in a branching sequence, according to their shared biological characteristics (for example, morphological or molecular; comparative genomics is discussed in Module 9.1). The more ancestral the character, the more likely it is to be present in a greater number and variety of organisms. A simple example using morphological characters is seen in Figure 2.2.9 (page 34). Starting with the most-shared characteristic, a vertebral column, which is assumed to be the most ancestral, taxa are added to the tree sequentially, ending with the least-shared characteristic, hair, at the top of the tree.

a Character table

characters \ taxa	seastar (outgroup)	lamprey	shark	frog	chicken	dog
hair	0	0	0	0	0	1
amniotic (shelled) egg	0	0	0	0	1	1
four walking legs	0	0	0	1	1	1
jaws	0	0	1	1	1	1
vertebral column (backbone)	0	1	1	1	1	1

b Phylogenetic tree

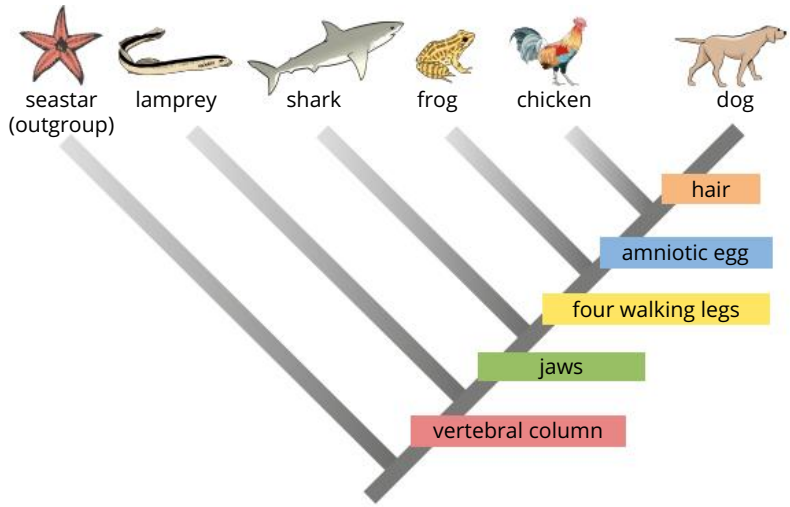


FIGURE 2.2.9 A simple phylogenetic tree built using morphological characters. (a) A character table lists different morphological characters and their presence (1) or absence (0) in each taxon is indicated. (b) Each taxon is sequentially added to the tree according to the presence of shared characters.

Each branch in the tree represents a change in character state (for example, DNA mutation) from the last common ancestor (Figure 2.2.10). The greater the number of nucleotide differences between sequences or taxa, the greater the distance between them in the tree, reflecting evolutionary relationships. The mutations that cause nucleotide differences are assumed to be equivalent for all species being compared. It is also hypothesised that the mutation rate is constant over time.

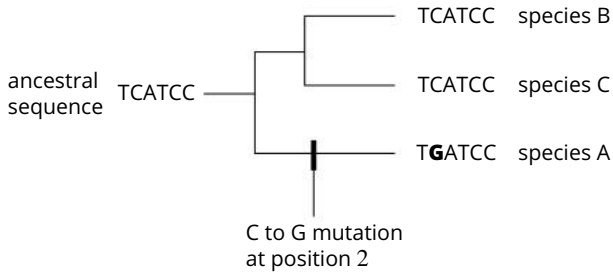


FIGURE 2.2.10 A phylogenetic tree built using DNA sequence data. In species A, a DNA mutation in the ancestral nucleotide has substituted C for G. This mutation results in a branch in the phylogenetic tree.

Most phylogenetic trees are now built using computational methods to generate more complex trees from large datasets. The parameters set for the computational methods are determined by the software user and some are outlined below.

One method that taxonomists use to construct phylogenetic trees is to draw the simplest possible tree to represent evolutionary relationships. This is known as **maximum parsimony** and is based on the principle of parsimony (also known as Occam's razor), which states that the simplest explanation is the one most likely to be correct. In the case of phylogenetic trees, the preferred tree (of the many produced) is the one that requires the fewest evolutionary changes to explain the variation (Figure 2.2.11). Although maximum parsimony is a popular method, there are also several other methods used to construct phylogenetic trees, such as maximum likelihood and minimum evolution.

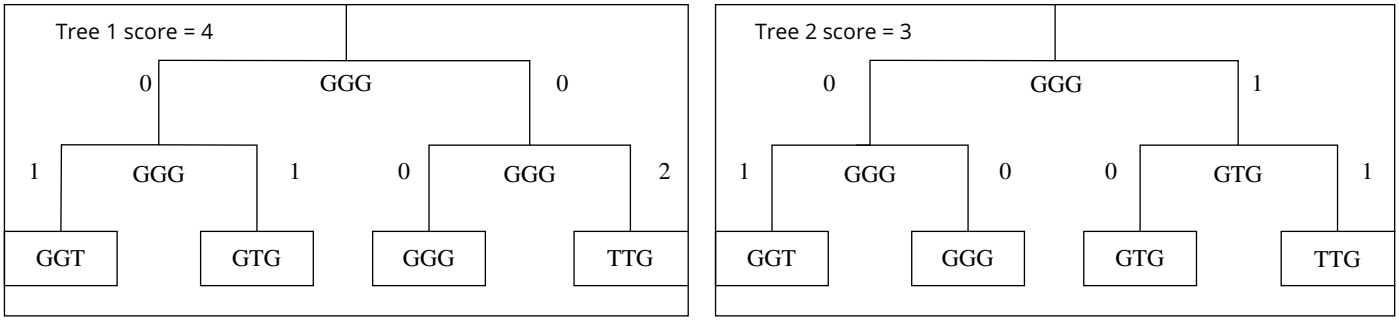


FIGURE 2.2.11 Using maximum parsimony to construct phylogenetic trees means selecting the simplest tree to explain the observed variation and evolutionary relationships between organisms. Tree 1 requires four evolutionary changes to explain the nucleotide change (G to T), but Tree 2 only requires three. Tree 2 provides a simpler, better explanation of the evolutionary relationships.

Parts of phylogenetic trees

As the name suggests, a phylogenetic tree is shaped like a tree with branches and leaves that extend from the **root** or hypothesised ancestral lineage. A lineage is all the species that are descendants of a common ancestor. Each line on the tree is called a **branch** and represents the hypothesised evolutionary path from a common ancestor. The end of each branch contains a scientific name and is called a **leaf**. The point at which two branches diverge is called a **node** (branch point or assumed bifurcation) and represents an inferred last common ancestor that the diverging groups shared.

An **outgroup** species is sometimes included in a phylogenetic tree. An outgroup is a taxonomic group that is possibly related to the other groups (**ingroups**) but is less closely related than any single one of the ingroups is to each other. An outgroup proposes a common ancestor with the ingroups that is older than the common ancestor of the ingroups. Outgroups are included to assess evolutionary relationships of those in the ingroup, relative to more distantly related taxa.

Pairs of taxa grouped together are called **sister taxa**. These are proposed to be the most closely related relative to other taxa in the tree. The most closely related (most recently diverged) taxa have the shortest branch lengths between them. In Figure 2.2.12, you can see that the closest relative of taxon C is taxon B, as that is the closest taxon following the branch from taxon C. Because no other taxa are proposed to be more closely related in the tree, taxa B and C are sister taxa. The next closest relative to taxon C is taxon A. Even though taxon D is listed under taxon C and they appear close to each other, if you follow the branch lengths between them, you can see that taxons C and D are actually quite distantly related.

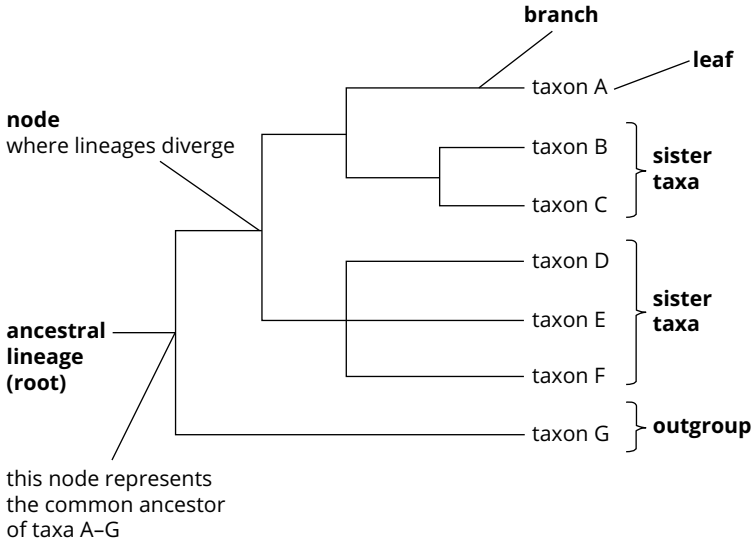


FIGURE 2.2.12 The components of a phylogenetic tree. The branches represent the evolutionary paths that each taxon has taken. Each of the nodes represents the divergence point of the taxa that have split off from it. Sister taxa (e.g. taxa B and C) are those that are the most closely related to one another. An outgroup (taxon G) has been included for comparison.

Each section of the phylogenetic tree is called a **clade**. A clade is a group of organisms that includes an ancestor and all the proposed descendants of that ancestor, also called a monophyletic group (Figure 2.2.13). The surmised order in which clades diverged from their common ancestor is represented by the order of the branching points (or nodes), with the oldest branching point closest to the root of the tree (the bottom in vertical trees and the left in horizontal trees) and the most recent branching point closest to the tips of the tree (the top in vertical trees and the right in horizontal trees) (Figure 2.2.14). As phylogenetic trees can have many different configurations, the order of the taxa at the tips of the tree does not always represent the order in which they diverged. Interpreting a phylogenetic tree and relatedness is relative to the hypothesised evolutionary relationships and taxa proposed and is not conclusive.

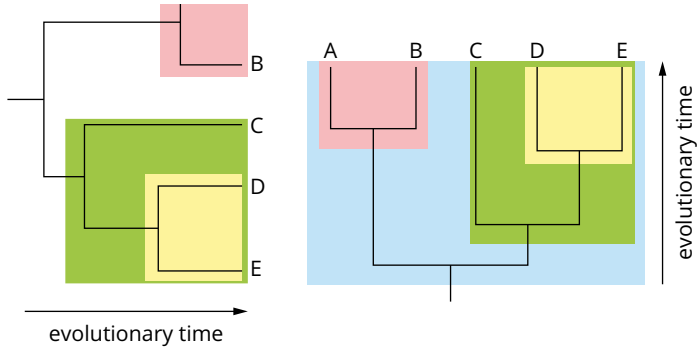


FIGURE 2.2.13 Phylogenetic trees consist of groups of taxa called clades. A clade includes a common ancestor and all its descendants. Each coloured square in these two trees represents a clade.

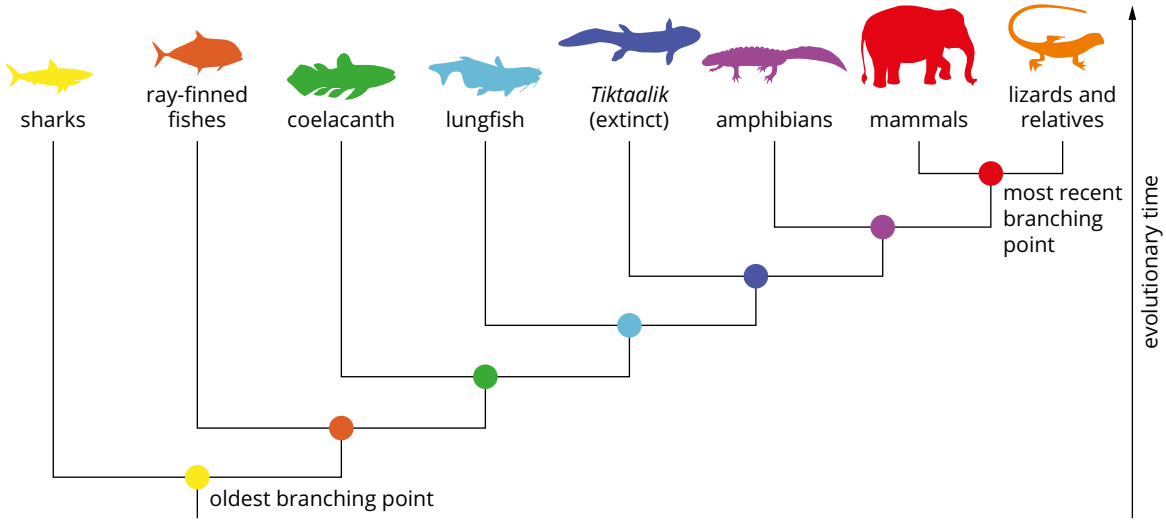


FIGURE 2.2.14 The order of the branching points (or nodes), from root to the tips of the tree (bottom to top in a vertical tree; left to right in a horizontal tree), represents the order in which clades diverged from one another.

The more closely related the taxa, the shorter the branch lengths between them will be. Shorter branch lengths in phylogenetic trees indicate the estimation of fewer evolutionary splits (divergence points) between the taxa and they are therefore less divergent and more closely related.

There are three ways in which taxa can be grouped within a phylogenetic tree (Figure 2.2.15).

- **Monophyletic groups** (one tribe) include a common ancestor and all of its descendants (a clade). For example, reptiles and birds are a monophyletic group (Figure 2.2.16). All the members of a monophyletic group can be removed from the tree with a single 'cut' (Figure 2.2.15).
- **Paraphyletic groups** (beside or surrounding the tribe) include a common ancestor and only some of its descendants (Figure 2.2.15). Although not taxonomically accurate, paraphyletic groups are useful for describing subsets of evolutionary groups. For example, dinosaurs are a paraphyletic group. Although birds are inferred to also be dinosaurs (both groups descended from a common ancestor), dinosaurs and reptiles are almost always referred to as a separate evolutionary group from birds (Figure 2.2.16).
- **Polyphyletic groups** (many tribes) include multiple descendants but do not include a common ancestor (Figure 2.2.15). This grouping is rarely used, but may group taxa on the basis of shared characteristics, such as the ability of birds and mammals to maintain their body temperature (Figure 2.2.16).

A monophyletic group contains a common ancestor and all its descendants. This group is also called a clade and is the only true taxonomic group.

WS 3.1.3

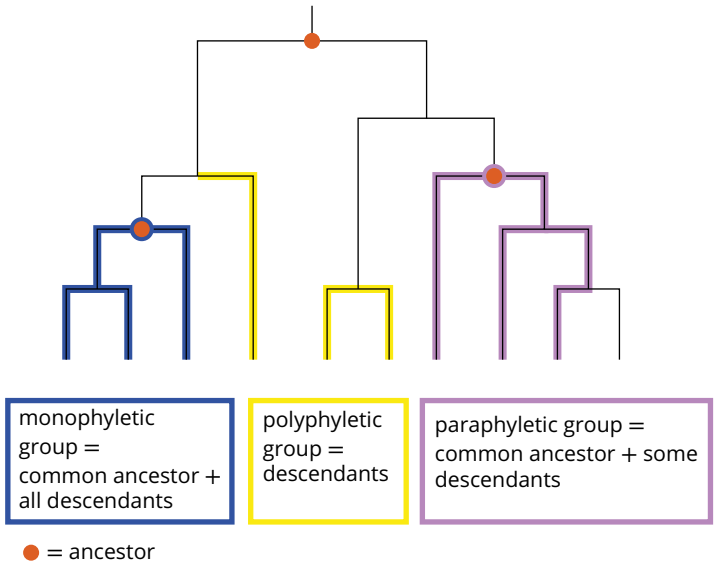


FIGURE 2.2.15 The three types of groupings within phylogenetic trees: monophyletic, paraphyletic and polyphyletic. Monophyletic groups are the only true taxonomic groups.

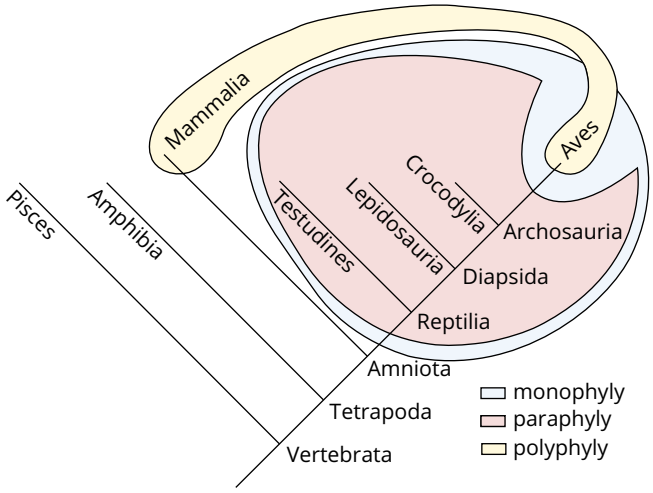


FIGURE 2.2.16 Monophyly, paraphyly and polyphyly represented in a phylogenetic tree. The blue group includes reptiles (Reptilia) and birds (Aves) and so is monophyletic (includes all descendants of a common ancestor). The pink group includes reptiles but excludes birds, so it is paraphyletic. Mammals (Mammalia) and birds can be grouped as warm-blooded animals (in yellow), but this group does not contain the common ancestor of these two descendants and so is polyphyletic.

Different forms of phylogenetic trees

Phylogenetic trees can be depicted in many different ways to show the same evolutionary relationships. Phylogenetic trees come in two main forms: **rooted trees** and **unrooted trees**. Both these tree types can also have scaled or unscaled branches. These trees are referred to as **cladograms** if they are unscaled and **phylograms** if they are scaled (Figure 2.2.17).

The branch lengths of a cladogram are not proportional to the amount of evolutionary divergence (nucleotide changes) between taxa. Phylogram branch lengths are proportional to the number of surmised nucleotide changes that have occurred in the lineage during divergence from the common ancestor and the total length of all the branches connecting two taxa in a phylogram is equal to the evolutionary divergence between them.

Both scaled and unscaled trees can be calibrated to a hypothesised timescale, which is determined by the software user, and so provide a visual representation inferring when, in evolutionary time, lineages diverged from one another.

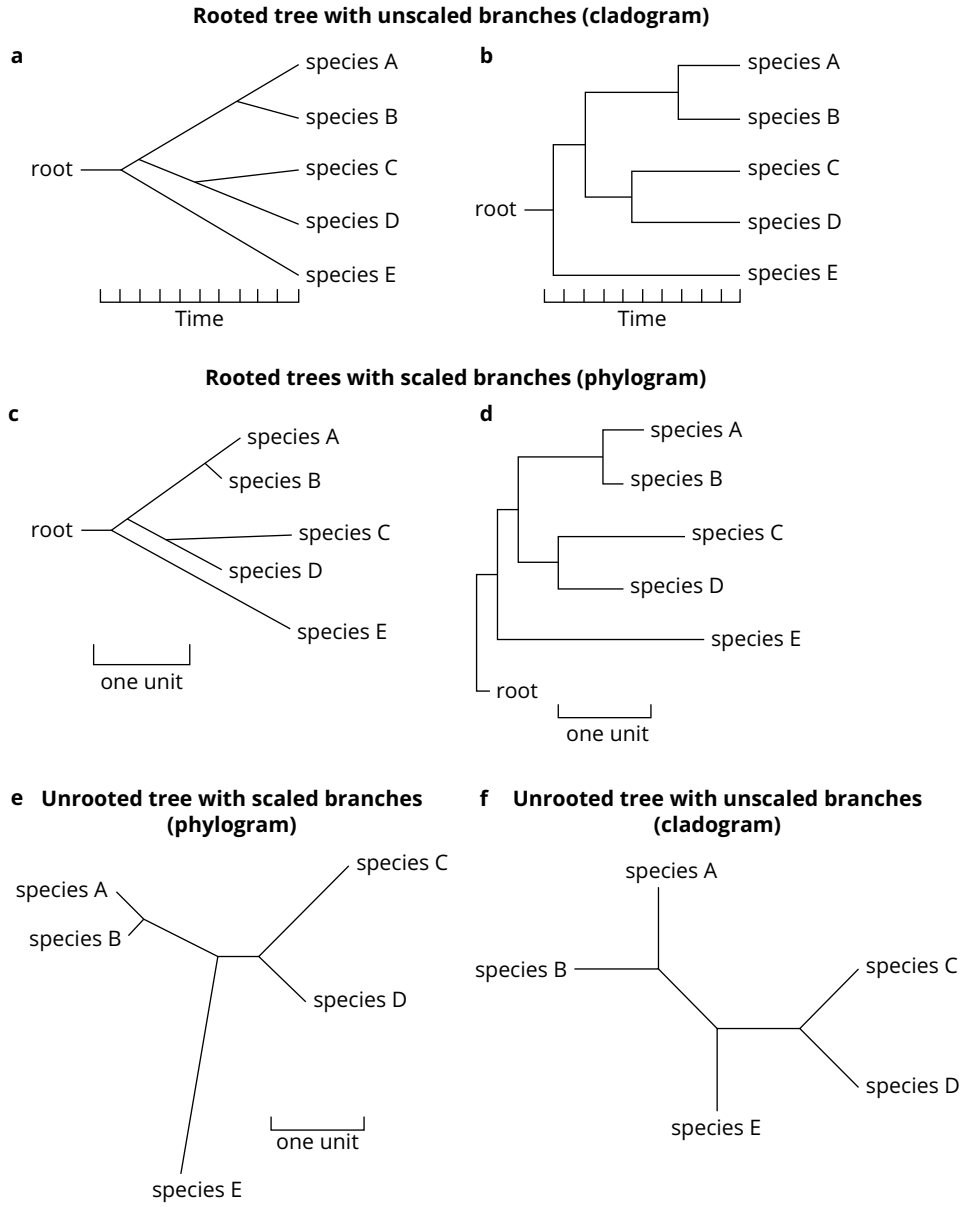


FIGURE 2.2.17 Two different ways of drawing rooted phylogenetic trees: (a, b) unscaled branches (cladograms) and (c, d) scaled branches (phylograms). The unscaled trees in (a) and (b) have been calibrated to a timescale. Two different ways of drawing unrooted phylogenetic trees: (e) scaled branches and (f) unscaled branches.

Phylogenetic trees may be presented horizontally or vertically, and branches may be diagonal or square (Figure 2.2.17). These different tree types and configurations may appear quite different even when representing the same evolutionary relationships. The branch points and their positions relative to one another are the parts of the tree that tell you the most about evolutionary relationships of the taxa and should remain the same even if the format of the tree is changed (Figures 2.2.17 and 2.2.18). In other words, trees that look quite different can represent the same evolutionary relationships. It is the order of branching points (nodes) from root to tip that is important, as this represents the order of evolutionary divergence of taxa.

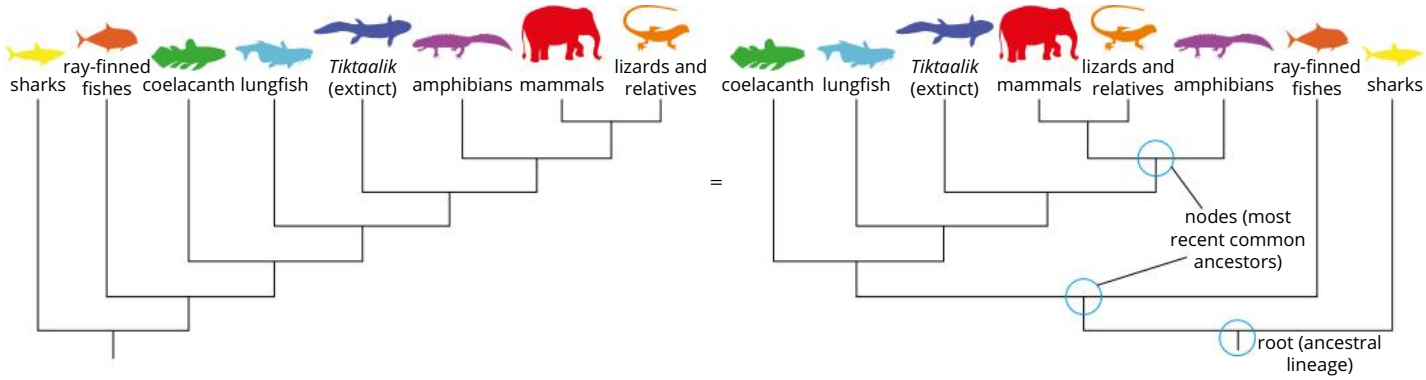


FIGURE 2.2.18 The same evolutionary relationships may be presented in different ways in phylogenetic trees. In these trees, the order of the taxa is different but the configuration of the branches connecting them remains the same.

Rooted trees

Rooted trees are drawn with the first branches coming from the base or 'trunk' of the tree. These may be drawn vertically (Figure 2.2.19), horizontally or in a circular format. The root represents the hypothesised common ancestor, or assumed common ancestor (when testing a hypothesis), of all taxa in the tree and the branches represent the inferred evolutionary path of those taxa over time. In comparison, unrooted trees depict evolutionary relationships between taxa in the tree but do not show their evolutionary path from a common ancestor.

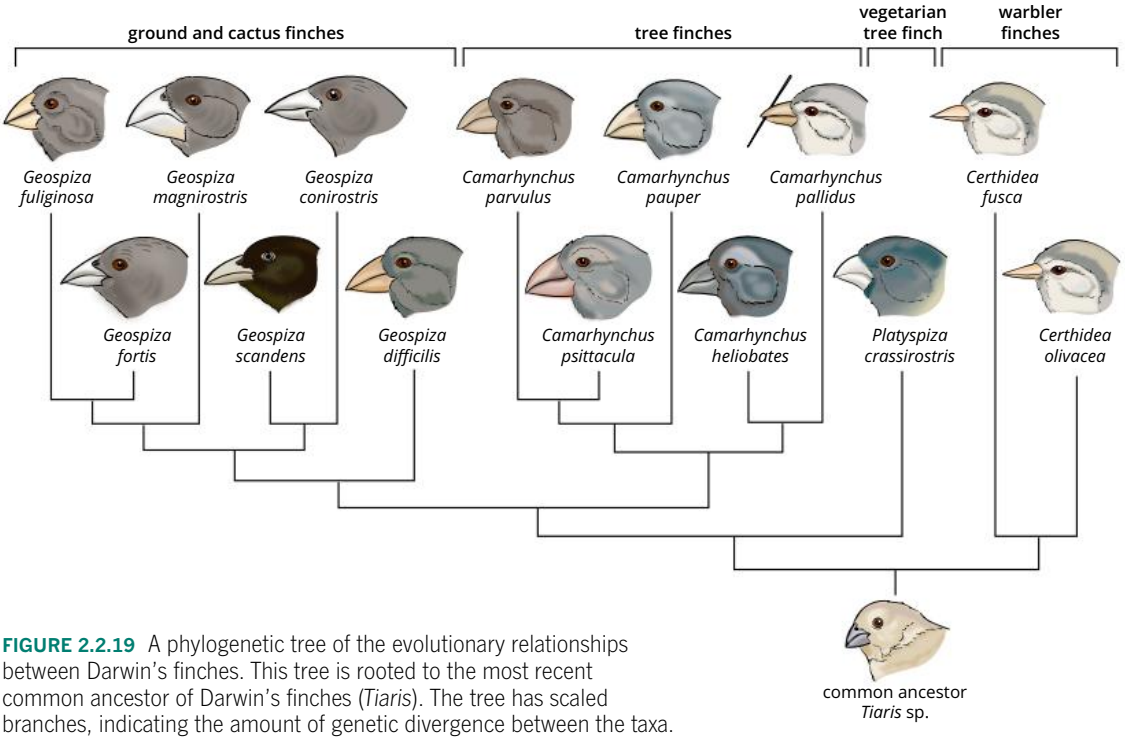


FIGURE 2.2.19 A phylogenetic tree of the evolutionary relationships between Darwin's finches. This tree is rooted to the most recent common ancestor of Darwin's finches (*Tiaris*). The tree has scaled branches, indicating the amount of genetic divergence between the taxa.

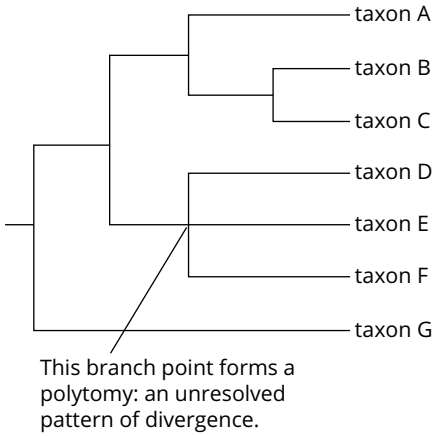


FIGURE 2.2.20 A rooted phylogenetic tree with a polytomy between taxa D, E and F.

In order to root a tree to a common ancestor and classify living organisms, an organism that is known to be related to the main group of interest is included in the phylogenetic analysis as an outgroup. The outgroup (warbler finches in Figure 2.2.19 on page 39) provides a hypothesised comparison point to assess where the main group of organisms (ingroup) sits in relation to other closely related taxa. The outgroup must be related to the ingroup in order to make meaningful comparisons, but not be more closely related than organisms in the ingroup are to one another. An outgroup can be selected by aligning DNA sequences to determine how closely related they are (assuming all lineages have undergone equivalent mutational changes and rates).

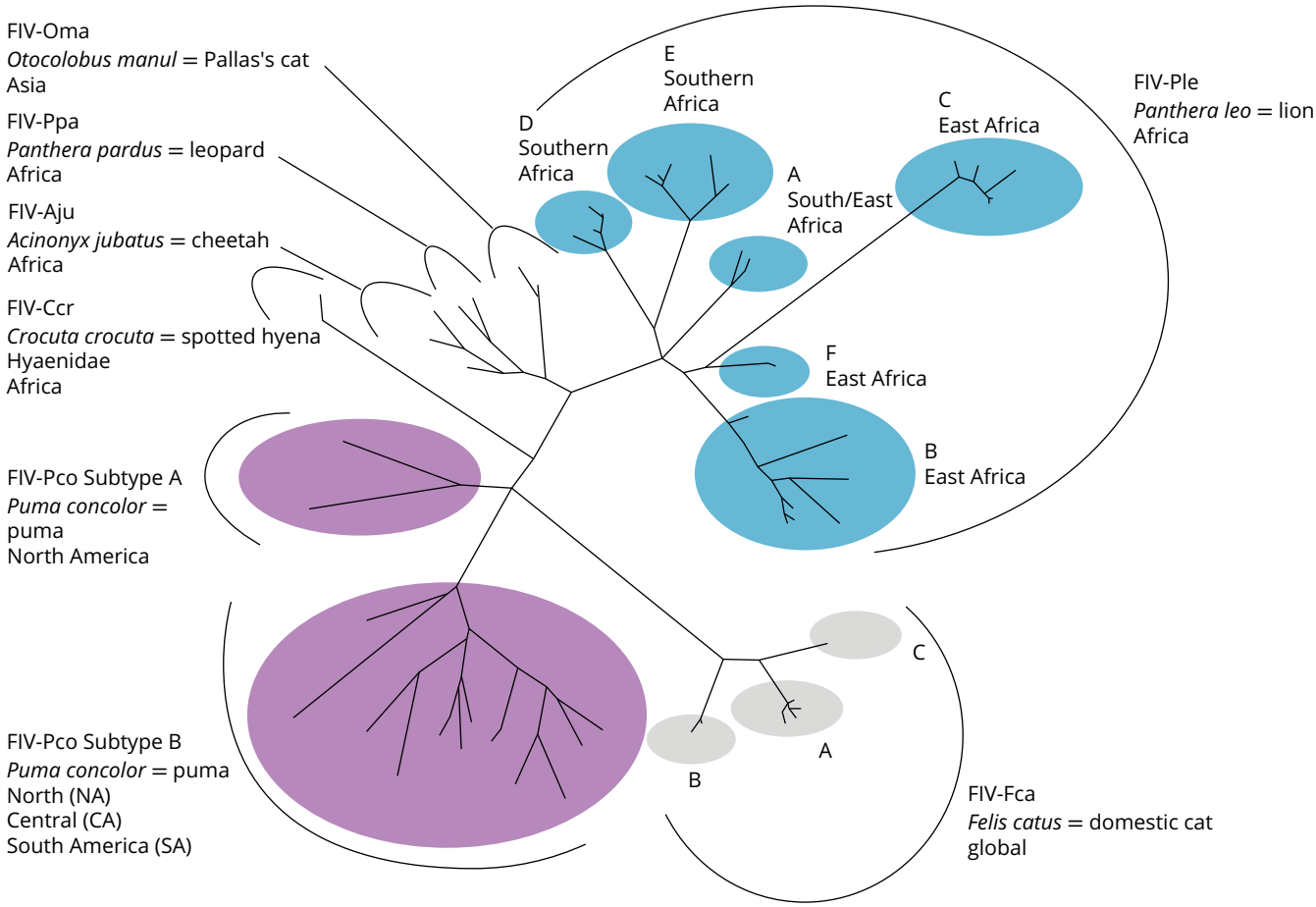
In phylograms the lengths of the branches connecting two organisms indicates the amount of genetic divergence between them.

Occasionally there will be nodes with more than two descendant lineages (like a garden rake). These nodes are called **polytomies** (Figure 2.2.20). Polytomies occur where there is not enough information to distinguish the order of evolution or when rapid speciation has occurred after evolutionary radiation in a new environment (Chapter 9). If rapid speciation has occurred, then all the daughter lineages will be closely related.

Unrooted phylogenetic trees

Unrooted phylogenetic trees do not include an ancestral root. Because the ancestor is not defined in unrooted trees, they often have a radial layout. These trees only indicate a relationship between the different leaf nodes. They do not indicate which node is the most ancestral. Like a rooted phylogram, unrooted trees can also have scaled branches where the distance between each leaf (along the branch lengths) is an indication of the amount of genetic divergence between the two groups of organisms. A greater distance suggests more time has passed since they shared a common ancestor (Figure 2.2.21).

FIGURE 2.2.21 An unrooted phylogenetic tree representing evolutionary relationships between subtypes of the feline immunodeficiency virus (FIV) from seven carnivore species. The common ancestor has not been defined in this tree, so it has a radial configuration. Branches are scaled to represent the genetic distance between the virus subtypes.



CASE STUDY 2.2.1

Applied phylogeny: The origin of SARS

In 2002 and 2003, there was worldwide panic about a previously unknown virus infecting humans and causing severe acute respiratory syndrome (SARS). Symptoms of the disease include fever, lethargy, sore throat, cough and shortness of breath. The outbreak started in southern China and quickly spread to Hong Kong, Canada, Singapore, Taiwan and Vietnam via international travellers. Between November 2002 and July 2003, the SARS outbreak caused more than 8000 people to become ill and 774 deaths (Figure 2.2.22).

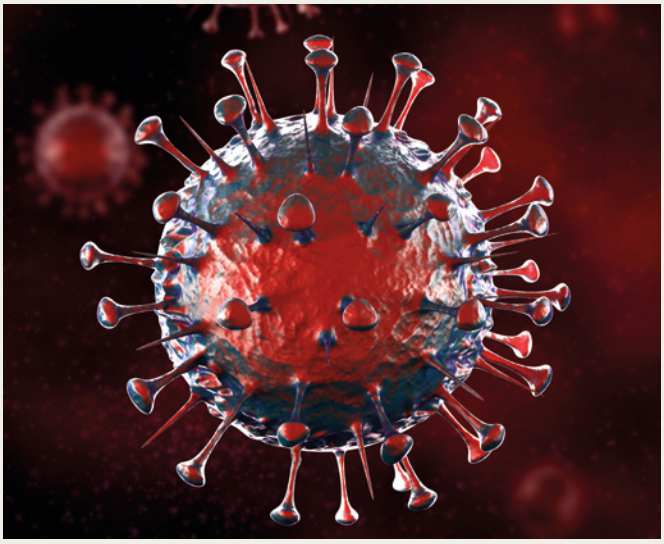


FIGURE 2.2.22 The severe acute respiratory syndrome corona virus.

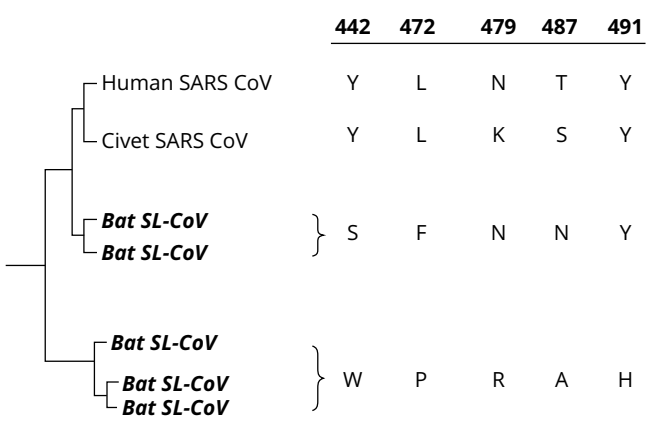


FIGURE 2.2.23 Scientists identified five key residual amino acids (denoted by the capital letters at positions 442, 472, 479, 487 and 491 on the protein strand) that could be used to establish a phylogenetic tree of the viral strain sourced from Chinese horseshoe bats. The scientists then compared these same key residues of the SARS corona virus to those found in palm civets and humans.

Viruses evolve rapidly, accumulating small changes in their DNA and RNA and forming new lineages. Much research was carried out to determine the origin of the SARS virus to improve our understanding of the disease and work towards minimising its impact. Scientists suspected that the virus originated from another species and had jumped hosts. There were two suspects: a cat-like mammal called a palm civet (used as a speciality food) and Chinese horseshoe bats that were found infected at a live-animal market in China.

Scientists used DNA hybridisation techniques to determine the number of differences in the RNA sequence of the viral genome. These differences were used to construct a phylogenetic tree for the virus using the genetic mutations of the virus strains (Figure 2.2.23). The phylogenetic analysis determined that the SARS virus originated in bats and then mutated before being defecated onto the plants and fruits eaten by the civets, enabling it to jump hosts and infect civets. The civet and human SARS viruses were found to be very similar and so the civet SARS is the most likely source of the human SARS virus.

Knowing the origin of a virus helps doctors understand emerging diseases and enables scientists to predict the infection patterns of future outbreaks.

Review

1. Identify the key residue differences between the human and civet strains of the SARS corona virus using the phylogenetic tree in Figure 2.2.23.
2. Determine whether the phylogenetic tree shown in Figure 2.2.23 provides definitive proof for the conclusion that the SARS corona virus that is present in civets originated from bats.
3. Interpret the data shown to predict which bat clade is most likely to spread a new strain of the corona virus to humans, assuming that the tree as drawn is highly probable.

CASE STUDY 2.2.2

The evolutionary uncertainty of ray-finned fish

Phylogenetic modelling of fish species reveals that there is uncertainty around evolutionary relationships of a group of ray-finned fish known as the percomorphs (Figure 2.2.24). You will notice that in Figure 2.2.25, the section of the phylogenetic tree (clade) with the percomorphs looks different from the rest of the tree. Instead of each node (branch point) having two lineages, the percomorph clade looks like a rake. There are many lineages, so this is a polytomy.

FIGURE 2.2.24 A foxface rabbitfish is an example of a ray-finned fish.

In this phylogenetic tree, there are two polytomies, one with three lineages (highlighted yellow) and the other with nine lineages (highlighted green). These polytomies indicate that there is not enough information to resolve the evolutionary relationships of lineages. The tree surmises that these lineages are closely related, but scientists can't be sure of the order in which they diverged from one another. From this phylogenetic tree, it is inferred that sticklebacks, spiny eels and guppies are closely related, but it is unknown which are most closely related.

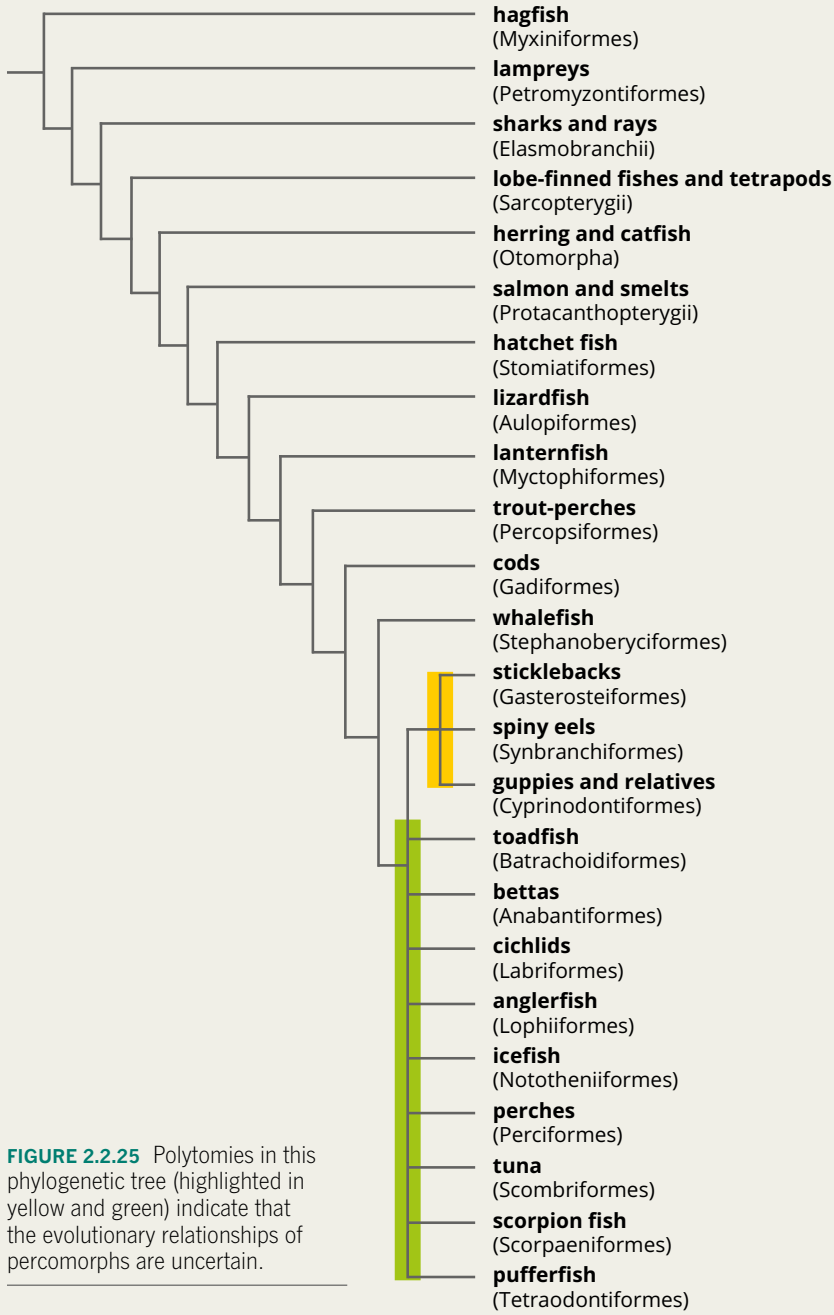


FIGURE 2.2.25 Polytomies in this phylogenetic tree (highlighted in yellow and green) indicate that the evolutionary relationships of percomorphs are uncertain.

Review

1. Identify the groups of fish in both the three-lineage polytomy and the nine-lineage polytomy.
2. New research is being conducted all the time. Researchers have applied the phylogenetic molecular techniques using mtDNA and made new assumptions with the percomorph clade from Figure 2.2.25. Research has resulted in the formation of 23 subgroups of percomorphs and 107 genera. Based on this research, identify whether or not the nine-rake polytomy has been addressed. Justify your response.
3. This research was published in 2012 and is the only one to date to investigate the percomorph clade polytomy. Conclude whether these findings form a model, theory, law, principle or fact (Module 1.1). Justify your conclusion.

2.2 Review

SUMMARY

- If two species have a similar set of proteins or DNA sequences, it is evidence that they shared a recent common ancestor.
- Changes in nucleotide sequences are caused by mutations.
- Mutations accumulate throughout the genome over time.
- Some genes accumulate mutations faster than others.
- Conserved genes accumulate mutations slowly.
- Mitochondrial DNA (mtDNA) is passed through the maternal line.
- Most cells contain many mitochondria and therefore copies of mtDNA are easier to obtain for analysis than nuclear DNA.
- DNA hybridisation involves the heating of DNA from two organisms so that they become single-stranded, and mixing them to allow the complementary strands of the two organisms to bond when cooled. The more similar the individual strands of DNA of the two species, the more hydrogen bonds will form between their complementary nitrogen bases, and the greater the amount of heat required to separate them.
- The formation of many hydrogen bonds means the DNA strands of the two organisms are very similar and share a recent common ancestor; a relatively high temperature will be required to separate the hybridised DNA strands.
- Phylogenetic trees show an inferred evolutionary relationship between different groups of organisms based on morphological and molecular homology.
- The branches and nodes of phylogenetic trees indicate common ancestry between organisms.
- A clade is a group of organisms that includes an ancestor and all the descendants of that ancestor.
- Phylogenetic trees can be used to classify organisms into clades (or various sized groups).
- Groups in phylogenetic trees can be described as monophyletic, paraphyletic or polyphyletic depending on their evolutionary relationships.
- Monophyletic groups are the only taxonomically viable group because they contain a common ancestor and all its descendants (a clade).
- Rooted phylogenetic trees can be used to indicate the length of time that has passed since organisms shared a common ancestor.
- Unrooted phylogenetic trees do not include an ancestral root, and only indicate the relationship between the different leaf nodes.

KEY QUESTIONS

Retrieval

1 Define phylogenetic tree.

Comprehension

2 Describe the scientific investigations that lead to the production of a phylogenetic tree.

3 Explain what an outgroup is and why it is included in some phylogenetic trees.

4 Summarise the four forms of phylogenetic tree.

Analysis

5 The table below shows the number of differences in the nucleotide sequence between each of the organisms. List the organisms in order from the one with the most distant common ancestor to the horse to the one with the most recent common ancestor to the horse. Explain how you came to this solution.

Differences in nucleotide sequence between selected species

	Human	Monkey	Dog	Horse	Donkey	Pig	Rabbit	Yeast
Human	0							
Monkey	1	0						
Dog	13	12	0					
Horse	17	16	10	0				
Donkey	16	15	8	1	0			
Pig	13	12	4	5	4	0		
Rabbit	12	11	6	11	10	6	0	
Yeast	66	65	66	68	67	67	67	0

continued over page

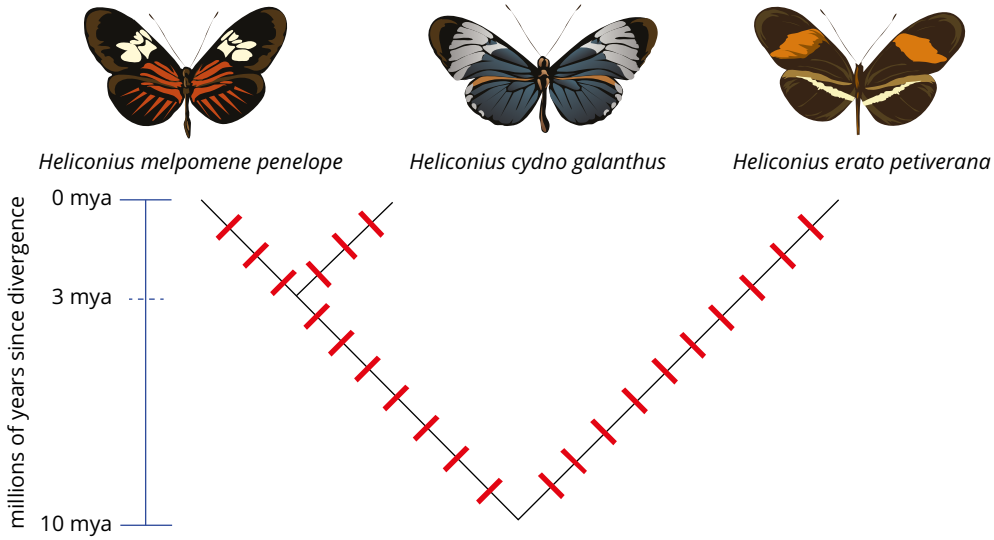
2.2 Review *continued*

6 The phylogenetic tree below depicts the divergence of three *Heliconius* butterflies from a common ancestor. The red markings on the tree represent a major nucleotide mutation.

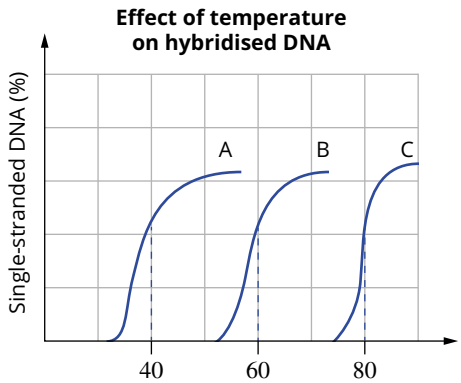
a Identify how long ago the most recent divergence was.

b Distinguish how many mutations *Heliconius erato petiverana* has accumulated since diverging from the most recent common ancestor.

c Calculate the assumed mutation rate (per million years) used to construct this phylogenetic tree.



7 A small section of DNA from a monkey was hybridised with DNA from species A, B and C. The hybridised DNA was then reheated to determine the temperature at which it became single-stranded. Determine which species is most closely related to the monkey and explain why, using the graph below.



8 Create a simple rooted cladogram based on cytochrome *c* differences of four species of mollusc using the data in the table below. Explain your placement of the branches.

	Species A	Species B	Species C	Species D
Species A	0	10	15	14
Species B	10	0	6	13
Species C	15	6	0	10
Species D	14	13	10	0

Question 9 refers to Figure 2.2.8 on page 33, where two phylogenetic trees are shown representing the evolutionary relationships of placental mammals. Tree A was constructed using morphological data and Tree B was constructed using molecular data.

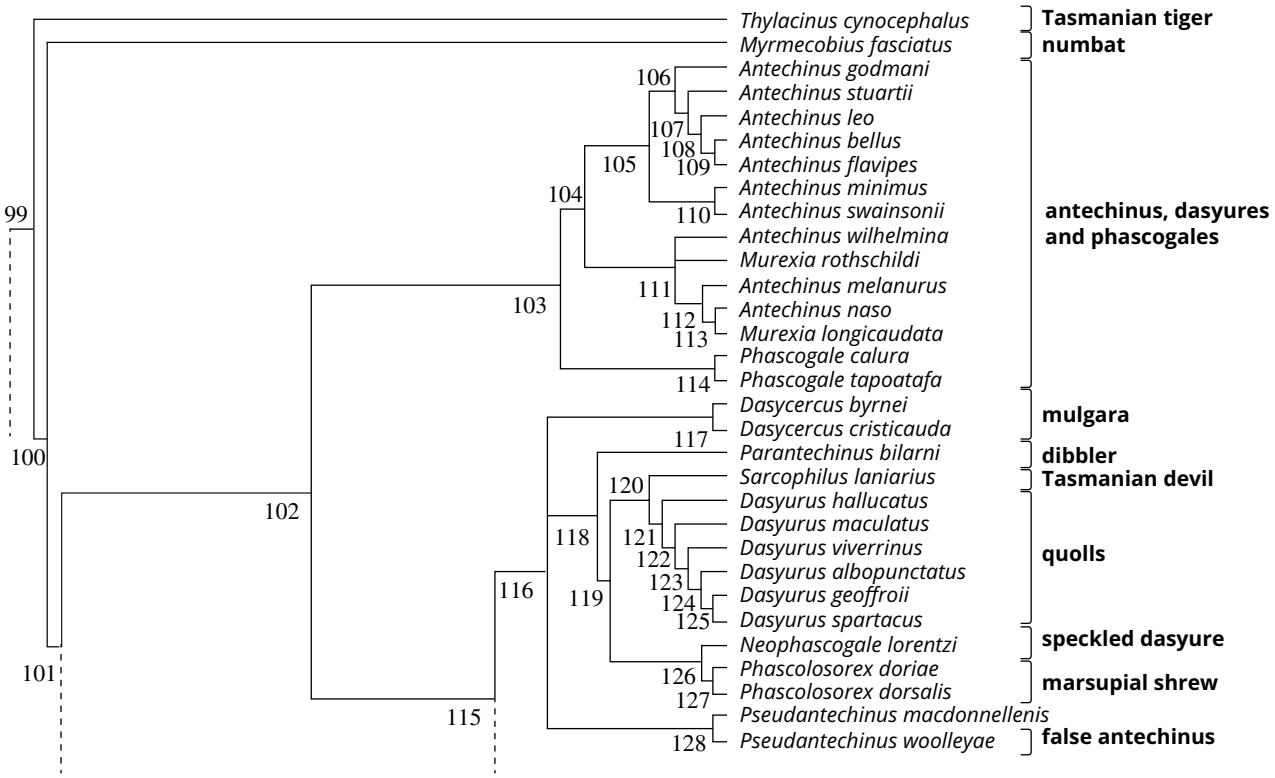
9 **a** Determine the two most closely related groups to primates on Tree B.

b Differentiate the grouping of the orders of placental mammals between both trees and distinguish the changes in orders.

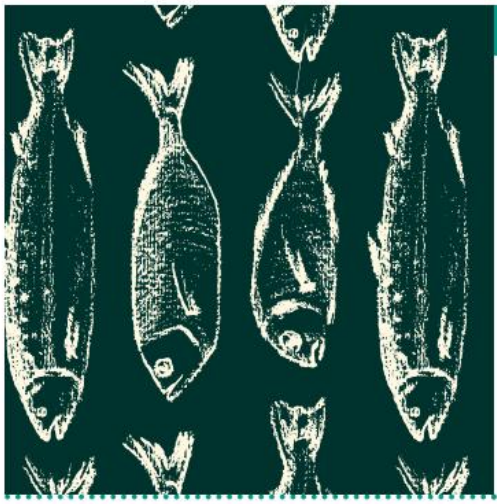
10 The diagram below shows a supertree cladogram for the carnivorous marsupial families Thyacinidae, Myrmecobiidae and Dasyuridae.

a Examine the taxonomic group of the Tasmanian devil and all the species that share a common ancestor.

b Distinguish the quolls from the Tasmanian devil and then from the Tasmanian tiger according to this cladogram.



2.3 Classification in practice



BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- describe the process of stratified sampling in terms of:
  - purpose (estimating population, density, distribution, environmental gradients and profiles, zonation, stratification)
  - site selection
  - choice of ecological surveying technique (quadrats, transects)
  - minimising bias (size and number of samples, random-number generators, counting criteria, calibrating equipment and noting associated precision)
  - methods of data presentation and analysis
- describe the use of dichotomous keys and multi-access keys for species identification.

So far in this chapter you have looked at the theory and processes involved in classifying organisms and ecosystems. And while satellite imagery and long-range magnetic sensing devices are important tools in mapping out bioregions and ecosystems, the most detailed and precise data comes from researchers in the field. Geologists are required to classify rock samples and mineral deposits. Meteorologists are needed to record local weather conditions such as humidity, rainfall and temperature. Biologists (which includes botanists, environmentalists, zoologists, entomologists, ornithologists, mycologists and microbiologists) will conduct **stratified sampling** techniques to identify the individual species and how they interact with the biotic and abiotic factors in that ecosystem. It is only when potentially endangered species and communities have been classified that we can begin to formulate appropriate management programs to conserve and protect our environment.

CLASSIFYING ECOSYSTEMS

Classifying an ecosystem in Queensland was described in Module 2.1. In this section we will be looking at the techniques used to conduct stratified sampling and, in doing so, identify the vegetative community (the third stage of ecosystem classification) and individual organisms and their distribution within the ecosystem.

Stratified sampling

Stratified sampling breaks a large population down into similar subgroups, or strata. In other words, the sampled population is partitioned into non-overlapping strata. Data samples are then randomly taken from each of the strata, with the confidence that each sample is more representative of the larger population. As each stratum is fairly homogenous, there is less variation in each dataset, which means a greater statistical precision in overall species composition of the ecosystem (Figure 2.3.1).

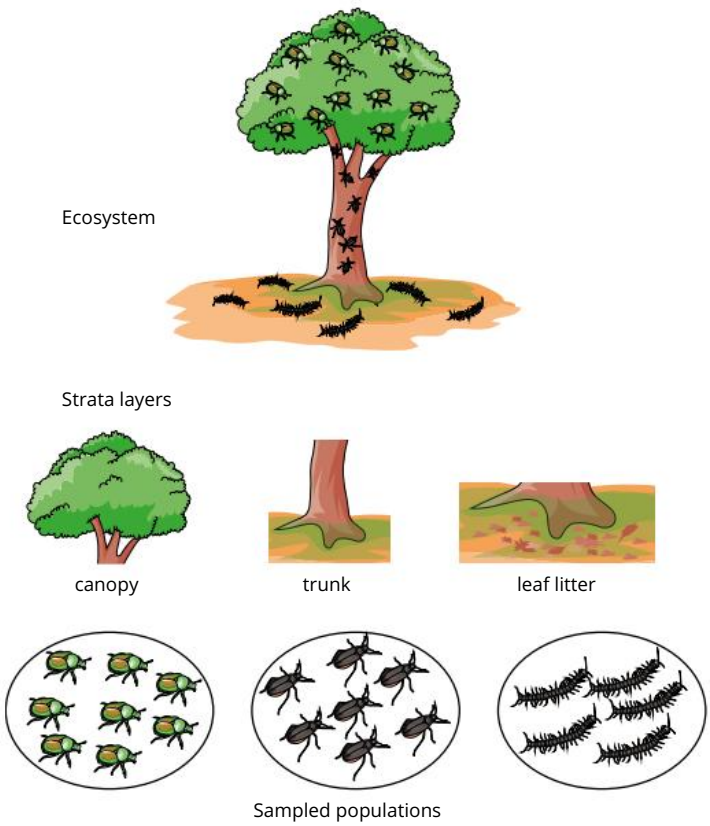


FIGURE 2.3.1 Ecosystems can be subdivided into various strata or homologous zones. Examples in this ecosystem could be zones of tree canopy, bark crevices or ground cover under the tree canopy.

As groups are homogenous, it allows researchers to estimate (through sampling) the population densities, zonation of species or the existence of environmental gradients (changes in the abiotic factors present, for example, moving from swampy ground to drier areas).

One example of stratified sampling could be students in a class sitting an exam on hormonal cycles. The different strata (or groups of similar students) could be based on gender or average grades. The results of the exam are a dataset. The data could indicate that one gender group had a better understanding of hormonal cycles, or that the complexity of the topic meant that only high-achieving students could pass.

Another scenario is soil samples that were taken at a research site in the Northern Territory to establish the species composition of the soil seedbank in open grassy areas and shrub areas. The seeds lie dormant in the soil, waiting for the right conditions to germinate. Stratified sampling techniques were used. Soil samples were taken and the soil was processed for species identification (Strata 1: soil). Belt transects were then made to establish the above-ground species composition (Strata 2: grassy areas and shrub areas) of this site.

That spring, a controlled burn-off was done. After the seeds germinated and the plants were growing, another bushfire swept through. If a second survey of the soil seedbank is done, the data should indicate which species has a germination strategy that allows it to survive multiple bushfires in one season. This species could have the ability to become the dominant species in a plant community (Figure 2.3.2).

FIGURE 2.3.2 Data collected from stratified sampling led to the strategy for elimination of bellyache bush (*Jatropha gossypiifolia*), a noxious weed that invades cattle stations in Northern Territory.

Sample site selection will vary according to the type of survey being conducted. A survey that samples all strata at the same time will be placed in areas that are representative of the vegetative community as a whole. Stratified sampling will focus on distinct subgroups of the ecosystem. Wherever sites are placed, they should be clearly marked and their location recorded so that further surveys can be carried out in the future to determine what changes have taken place over time.

General advantages of stratified sampling

With careful partitioning of populations into chosen strata, the more homogenous the strata populations will be and the less error there will be in the sampling data. Variation will be lower and, with enough data samples, standard deviation and standard error become highly reliable. Calculated parameters such as population mean, density, distribution, zonation and environmental gradients and profiles will include fewer errors and will be more representative of true values. Using stratified sampling, observations and measurements of relationships between environmental variables and parameters of interest may be more statistically significant or realistic (valid and reliable) in their application. Done well, this will offer greater reliability in estimating population parameters outside the sampled area.

Ecological surveying techniques

Transects

Transects are lines or belts set out through the chosen study area as a guide for recording which species are present in a community. They provide a useful method for showing changes in the abundance of a particular species in response to changes in one or more physical variables along a gradient. Key abiotic factors that affect species distribution include slope, altitude, temperature and soil moisture content.

A **line transect** is made by running out a tape measure or marked line across the area to be sampled (Figure 2.3.3). The general recommended length used in forests in Queensland is 50 m, with a 5 m wide observation strip on each side of the line. A strip this size can be correlated with imagery taken by LANDSAT satellites. The observer systematically works along the line, recording the name and position of each plant that the line passes over, under or through. The data is recorded on paper on a corresponding line drawn to scale.

A **belt transect** is similar to a line transect except that all the vegetation between two parallel lines is recorded. A transect can also be used as a location line for quadrats, in which the cover of each species can be estimated.

A **profile diagram** is a scale drawing of the profile or side view (also called the elevation) of the vegetation along a line. It may also show the shape of the land surface and details of soil type. Profile diagrams show the horizontal layers or strata of the vegetation. Well-drawn profiles are often useful for making qualitative comparisons between different plant communities. Care needs to be taken with the choice of scales on the axes so that the drawing is not too distorted (Figure 2.3.3).

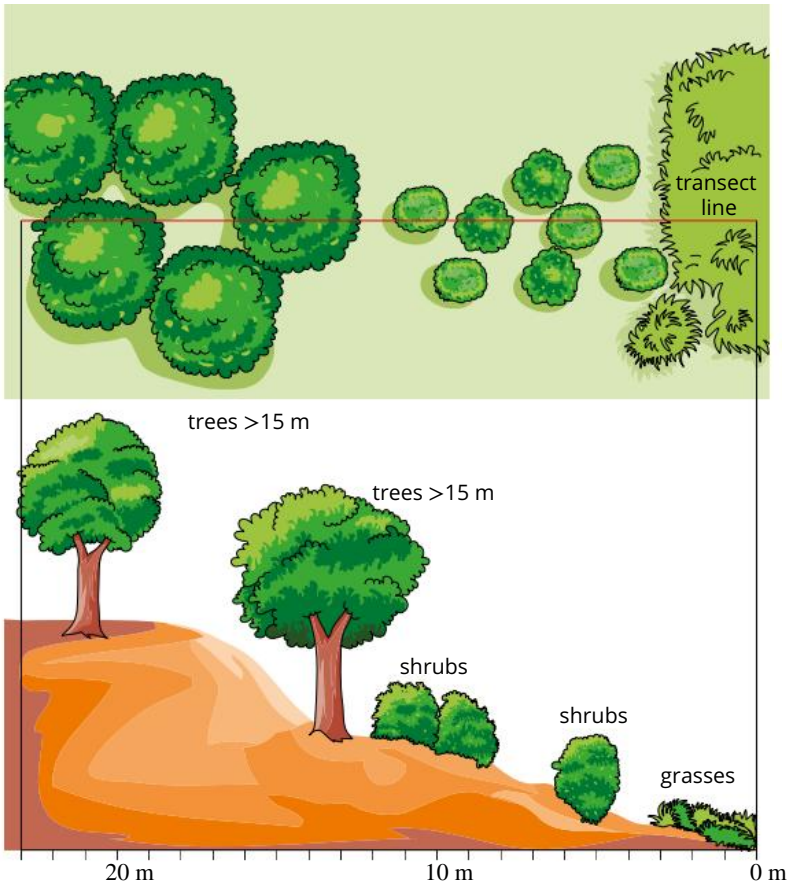


FIGURE 2.3.3 Transect line and profile diagram showing plant types and slope.

Data collection and presentation

Data collection should always be comprehensive, as it is often difficult to return to remote areas, or even have the time to do so. Data can be recorded like the example in Table 2.3.1.

TABLE 2.3.1 Transect 1 data

Transect mark (m)	Vegetation type	Canopy cover (%)	Substrate type	Slope (%) (rise/run ×100)	Soil pH	Air temp. (°C)	Soil temp. (°C)	Notes
0	short grass and herbs	• grass 80 • herb 5 • bare soil 15	clay soil	2	6.5	27	23	samples T1.0.1 grass, T1.0.2 herb
1	forbs to 50 cm and grass	• forbs 30 • grass 60 • bare soil 10	clay soil	4	6.5	27	23	sample T1.0.3 forbs
18	shrubs 2–4 m and grass	• shrub 75 • grass 5 • bare soil 20	clay loam	10	6.9	26	21	photo 15 shrub flower and leaf/ whole plant

The headings you select for your table are determined by the type of ecosystem you are surveying. The example in Table 2.3.1 is suitable for a dry land vegetative community. If you were surveying a freshwater stream community, you would include speed of current, depth of water, surface and bottom temperatures, for example.

Species identification is sometimes not possible in the field, so specimen samples are usually taken and labelled with their unique sample number and studied in the lab. The location and position of each specimen that was collected must be recorded, for example, in a log book. Details recorded should include the transect or quadrat position, date of collection and a simple description of the specimen or where it was found (for example, 'pale flat insect under small log' or 'small pink-flowered herb'). If it is illegal to remove samples from the survey area, clear photographs must be taken, or detailed line drawings made in a logbook or journal. Data from this type of survey is required to identify the type of vegetative community using Specht's classification method.

Quadrats

A **quadrat** is a square, rectangular or circular frame of convenient size, used to mark out an area in which the vegetation is to be sampled. The shape and size of the quadrat depends on the type of vegetation. A square with 50 cm sides would be suitable for sampling a lawn or moss and lichens on a tree trunk, whereas a square with 10 m sides may be used for sampling trees in a forest (Figure 2.3.4).

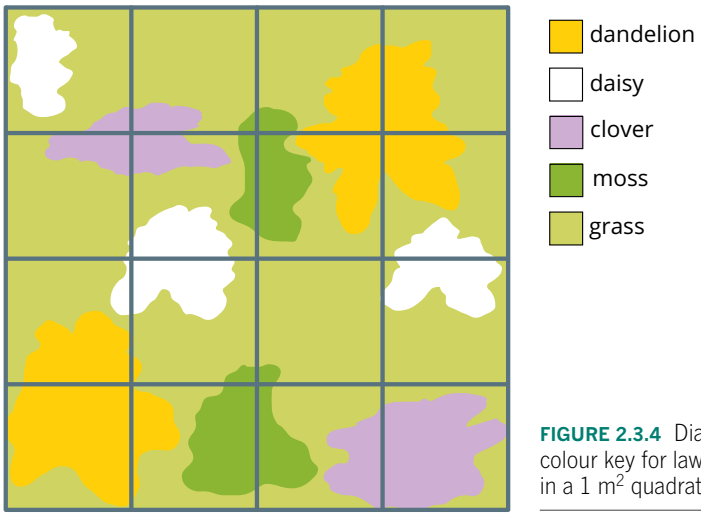


FIGURE 2.3.4 Diagram showing colour key for lawn plant life found in a 1 m^2 quadrat.

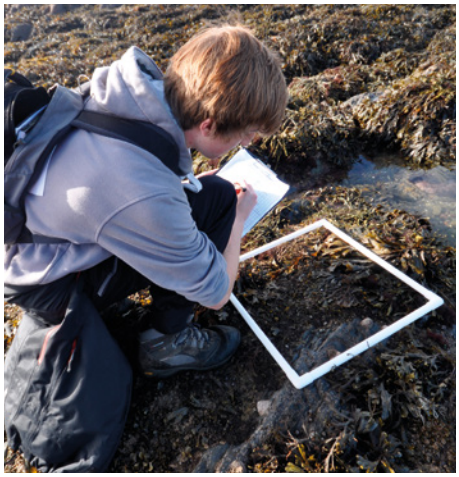


FIGURE 2.3.5 Student using a quadrat to determine species composition of seaweed on a rocky shore.

Quadrats may be located randomly over the area being sampled or at regular intervals along a transect line or as a grid pattern. When quadrats are used to sample vegetation, a sufficient number must be used to obtain representative data. First, a list is compiled of all the plants contained in each quadrat (you can collect a sample or take a photograph for identification later). Repeating this for several quadrats should provide a fairly comprehensive list of the species present in the area (Figure 2.3.5).

Quadrats can also be used to give an estimate of the abundance of one or more of the species. Two ways of measuring abundance are density and cover. Density is the number of a particular plant species in a given area. Cover is the percentage of ground covered by a particular plant species in a given area. This data is particularly important for using Specht's method of identifying a vegetative community.

As quadrats are a representative sample, the number of quadrat samples poses a limitation. It is not possible to sample the entire ecosystem, therefore any representative survey technique, such as quadrats, provide limited data of the entire ecosystem. All ecological science research is limited as it is rarely possible to survey an entire ecosystem. It is important that the representative samples do not skew the data causing systematic error. It is also important that a sufficient number of samples are surveyed to minimise random error.

Minimising bias

There is a strong tendency for all of us to be drawn to places that look promising. In terms of site selection, researchers might prefer to sample places that have a greater variation of species or look greener and lusher; however, this may not be truly representative of the whole site. Randomised sampling techniques are designed to reduce this bias as much as possible. Biased sampling can lead to statistically significant differences between the data samples collected and the actual species composition of the site, skewing the results of the research.

There are various methods employed by researchers to minimise this bias.

1 Law of large numbers: The greater the number of samples taken, the greater the probability that sample mean (or average) is representative of the community as a whole.

2 Random-number generators: Bias in site selection for quadrat sampling or positioning of transect lines can be minimised by the application of a grid overlay on the survey area. A random-number generator is then used to select grid coordinates for the start and direction of transect lines or for the placement of quadrats.

3 Counting criteria: These are the parameters used for counting organisms within a quadrat. For example, 'Count any plant that is even partially inside a quadrat' or 'Do not count a plant unless the base of the plant is completely inside the quadrat, even though the top of the plant is outside the quadrat'. Counting requires consistent rules across the whole of the sampling area, otherwise there will be a built-in error from the variation that will creep into the results.

4 **Calibration** of equipment: The use of data loggers is an industry standard these days, but the quality of the data collected is very dependent on whether or not the devices have been serviced and correctly calibrated. The precision of each type of data sensor will vary according to its use. For example, pH and temperature (°C) may be suitably measured to one decimal place; however, conductivity (mS) may require a precision of three decimal places. Before each field excursion, all sensors should be cleaned and checked for accuracy. After every excursion, they should be cleaned and checked for damage. Understanding the biotic and abiotic relationships in an ecosystem, or the tolerance range of a particular organism, is intrinsically dependent upon the accuracy of the data collected (Figure 2.3.6).

FIGURE 2.3.6 Digital data loggers are excellent for collecting environmental data such as light, soil moisture and pH. However, the accuracy of the information is very dependent on both the calibration and maintenance of the equipment being used.

Data analysis

After all the data has been collected and collated in the form of tables, transect profiles and diagrams, the data can be used to classify the ecosystem, identify habitats within the ecosystem and identify relationships between organisms. The data can be used to look at population ratios for predator–prey relationships or the preferred habitats of rare and endangered species (Figure 2.3.7).

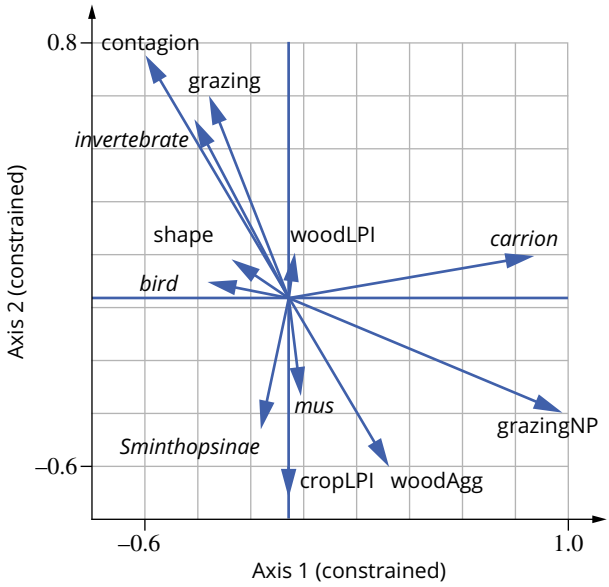


FIGURE 2.3.7 A redundancy analysis triplot of red fox prey scat composition against various landscape types in the Brigalow Belt South bioregion of Queensland. This representation of the data highlights the vulnerability of native mammals as substitute prey for foxes when there is a scarcity of European rabbits.

Data collected over time can show a rate of succession of species in the recolonisation of an area after a catastrophic event (such as a bushfire or volcanic eruption). Data analysis is an essential tool in the formation of management policies for the conservation and preservation of ecosystems, so the data must be reliable.

IDENTIFYING SPECIES

You have already been introduced to the concept of classifying species in Module 2.1, but to fully understand the interactions in an ecosystem we need to individually identify all the species present. Researchers and enthusiasts use several methods. They may take a sample or image of the organism and send it to an expert in that field. Other enthusiasts will look at pictures in a book, an app, website or some form of simple identification guide. This is quite suitable when there are a limited range of species in that type of organism. However, it becomes difficult when someone has no idea of what type of plant or fungus, or reptile or bird they are looking at. Queensland has more than 9400 plant species and more than 1000 known species of fungus, so more precise means of identification are required.

Dichotomous keys

Dichotomous keys are tools constructed by biologists to help people identify and distinguish between organisms. Dichotomous keys use a series of pairs of characteristics to subdivide larger groups into smaller groups. The process continues until each organism or small group of organisms (perhaps several related species in one genus) can be distinguished from the others.

The first step in using a dichotomous key is to find one that suits your purpose. Often the identity of an organism is known at some level. You would have no difficulty determining that you have collected an insect or flowering plant. Some keys help identify the broad groups of class, order or family and keys to identify individual species. Some are restricted to certain geographical areas and others are restricted to particular groups of organisms.

The language used in a dichotomous key can be a limiting factor for the user. Keys used by researchers and professionals contain taxonomic descriptors that require tertiary training to understand. Other keys use everyday language as they are targeted at enthusiasts or high school students trying to establish species relationships in an ecosystem.

Dichotomous key in action

Dichotomous keys can be used to identify an organism to the level of species. You can use the key shown here to find out the species of a specimen of plant known as *Banksia* (Figure 2.3.8).

Begin at the top of Table 2.3.2 with the statements labelled 1a and 1b. Choose which of these matches your specimen best. For example, if your specimen has leaves less than 4 cm wide then it matches 1b, and the key tells you to go to 2. You now have to choose between 2a and 2b (note the terminology used). If your specimen matches 2b, then it is *Banksia serrata*.

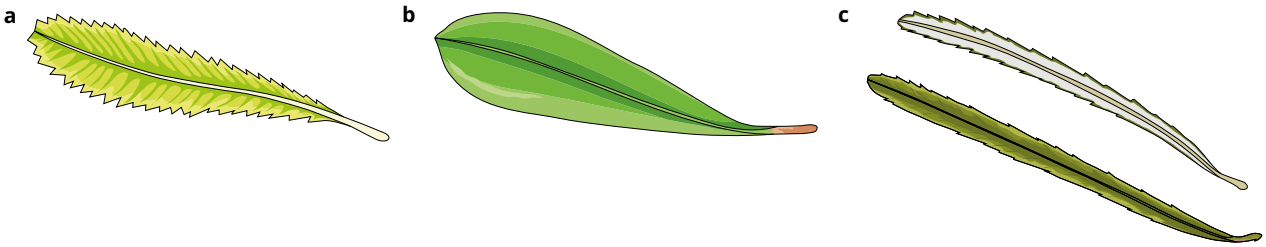


FIGURE 2.3.8 (a) *Banksia serrata*. (b) *Banksia integrifolia*. (c) *Banksia spinulosa*.

TABLE 2.3.2 A dichotomous key to the common species of *Banksia* in south-eastern Queensland

1a	leaves usually more than 4 cm broad	*Banksia robur*
1b	leaves usually less than 4 cm broad	2
2a	under surface of leaves densely covered with short matted hairs (tomentose)	3
2b	leaves lacking hairs on the under surface (glabrous)	*Banksia serrata*
3a	usually a shrub 3–5 m	4
3b	usually a tree to 25 m	*Banksia integrifolia*
4a	leaf stalk (petiole) usually 2.5–7 mm long	*Banksia oblongifolia*
4b	leaf stalk (petiole) usually 1–3 mm long and leaf margin rolled downwards	*Banksia spinulosa*

Groups of arthropods

We are all familiar with some of the different groups of the phylum Arthropoda. We can probably distinguish a spider from an insect or a crustacean, perhaps even a millipede from a centipede. This identification needs to be done in a systematic way so that unknown arthropods can be named. A simple branching key is shown in Figure 2.3.9. You start on the left-hand side, choosing the alternative that best fits a particular specimen For example, does it have paired appendages on most body segments or are they lacking? Continue to work through the appropriate pairs of statements.

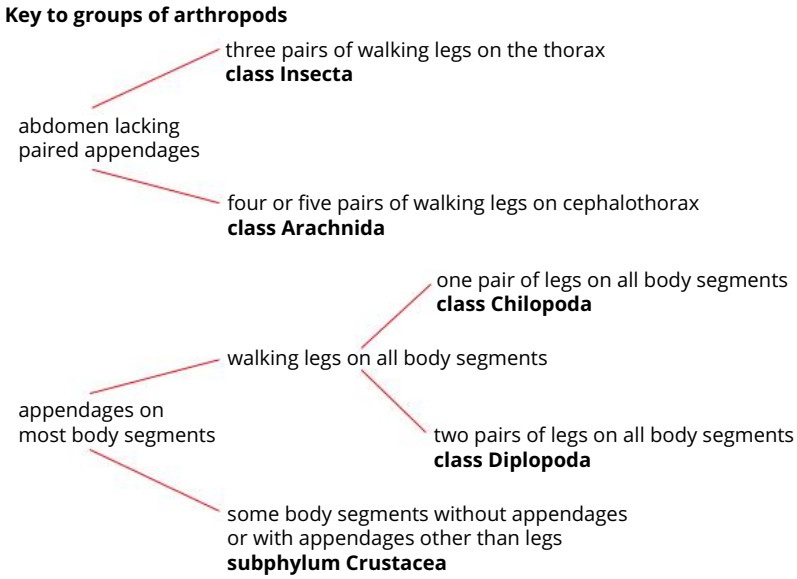


FIGURE 2.3.9 A key to groups of arthropods.

Multi-access keys

Computer-based **multi-access keys** (also known as matrix keys) are not restricted by the order of progression in the way dichotomous keys are. Multi-access keys allow you to select any feature of the specimen and progress through the key in any order you wish. Multi-access keys are built using a database of characters (identifiable features of the taxon specimens) and states. For example, an identifiable feature of grass is how the grass seeds grow on the plant. The character could be 'Seed occurrence' and the state could be 'Seeds are close together', 'Seeds are spaced' and 'Seeds are single on a long stem' with images of each state as part of the key (Figure 2.3.10).

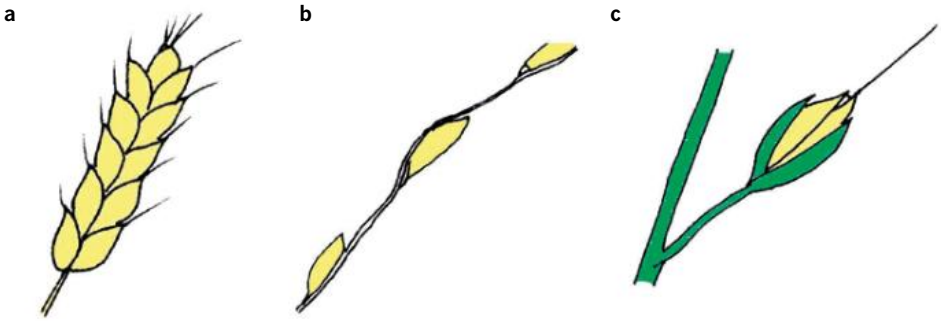


FIGURE 2.3.10 A multi-access key that uses the placement of grass seeds as an identification feature. (a) Seeds are close together. (b) Seeds are spaced. (c) Seeds are single on a long stem.

When first opened, a multi-access key will show all the species that can be identified using this particular key. As various states are chosen, species that do not have these traits are removed from the selection. The user continues to identify different features until a few, or a single, species remains. The advantage of a multi-access key is that you choose features that are obvious on your specimen sample, making identification much easier than with a dichotomous key.

WS 3.1.2

SCIENCE AS A HUMAN ENDEAVOUR

From a distance...

The LANDSAT 8 satellite (Figure 2.3.11) was launched on 11 February 2013. It carries two very important scientific instruments: the Operational Land Imager and the Thermal Infrared Sensor. These two sensors provide seasonal coverage of the global landmass at a spatial resolution of 30 m (visible, near infra-red [NIR] and short-wave infra-red [SWIR]); 100 m (thermal) and 15 m (panchromatic). Such detail, correlated with on-the-ground surveys, allows scientists to accurately map the changes in our ecosystems due to urban growth, deforestation and agriculture.

Since the first LANDSAT satellite was launched in 1972, their images have been used to map faults and fracture zones and identify volcanic and tectonic activity. LANDSAT images have also been used to monitor the seasonal changes in the Arctic ice pack, and have provided accurate data about the receding of the world's glaciers and the ice fields of Antarctica.

Having free access to LANDSAT imagery helps scientists understand the effect that changes in climate, human harnessing of freshwater resources and sedimentation spumes from river mouths have on our environment. It provides policymakers with data about which land areas need to be conserved in order to protect natural resources, such as the Great Barrier Reef and coastal mangrove forests.

Observations from the Australian Bureau of Meteorology show record-high night and day temperatures, combined with a summer monsoon period that had less than 41% of the regular rainfall and a 20 cm drop in local sea levels that lasted for a month. The Gulf of Carpentaria is a large, shallow body of water that is usually subjected annually to cyclones. Mangrove forests fortify and buffer the coastline against tidal and wave action and help to filter sediments from water. They extend the coastline and act as nurseries for many species of fish and crustaceans that are important to our fishing industry. LANDSAT imagery (Figure 2.3.12) showed extensive die-off of mangroves along the Gulf coastline.

FIGURE 2.3.11 LANDSAT 8 passing over the Gulf Coast of the USA.

FIGURE 2.3.12 Satellite imagery reveals a severe die-off of mangroves along Australia's northern coast. The data indicates that more than 7000 hectares of mangroves have died. These natural-colour images were acquired on (a) 15 July 2014 and (b) 20 July 2016 by the Operational Land Imager on LANDSAT 8. They capture the extent of mangrove die-offs on a strip of beach along the Gulf of Carpentaria.

Review

On the night of 15 February 2017, tropical cyclone Alfred formed in the Gulf of Carpentaria near Borroloola, Northern Territory. Alfred was a Category 1 cyclone, with maximum wind gusts of 85 km h^{-1}. It crossed the coastline in the early afternoon of 21 February 2017 before downgrading to a rain depression.

Tropical cyclone Alfred was the first tropical cyclone to form in the Gulf since the loss of mangroves on the coastline.

1. Predict what the possible impact of tropical cyclone Alfred on the exposed coastline might have been after five days of buffeting by wind and waves.
2. Considering that the last published LANDSAT image was from July 2016, identify what action local environmentalists should have taken after the 2016–17 cyclone season.

2.3 Review

SUMMARY

- Stratified sampling breaks complex ecosystems into homogenous subgroups or stratum.
- Using subgroups results in less variance and greater precision in data sampling of an ecosystem.
- The location of sample sites should be carefully recorded so that further surveys may be carried out in the same place to log the change in the ecosystem and its population dynamics over time.
- Line and belt transects are two common means of surveying ecosystems and can be used to construct a landscape profile.
- Quadrats are used for sampling the species distribution and composition of a survey site.
- Human bias is reduced in surveys by utilising the law of large numbers, random-number generators (for quadrat or transect placement), setting parameters when counting species in a quadrat and keeping precision equipment in a state of good repair and correctly calibrated.
- Analysis of collected data is an essential tool when developing a management plan for a region.
- Identification of organisms can be carried out to different taxa levels by using dichotomous keys or multi-access keys.

KEY QUESTIONS

Retrieval

1 State the advantage of stratified sampling over whole ecosystem sampling.

2 List four methods by which scientists can minimise bias in their randomised sampling of an ecosystem.

3 Describe the difference between a dichotomous key and a multi-access key.

Comprehension

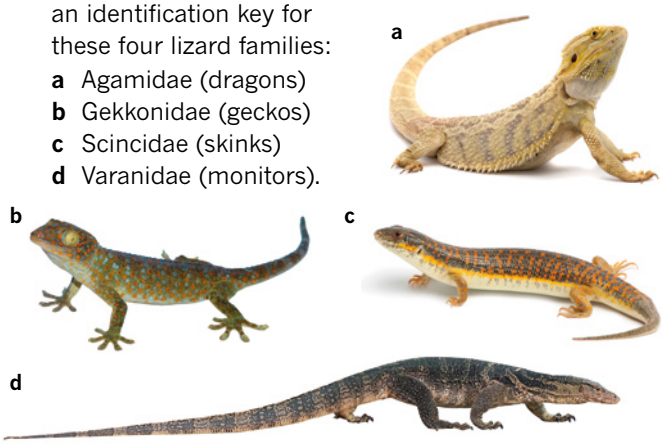
4 You are to conduct a 10 m long transect through some rock pools at Burleigh headland. You will be using a 0.25 m × 0.25 m quadrat at 1 m intervals along and on both sides of the transect line. Explain the headings that you would use in your data collection table.

5 Depict the key to *Banksia* (Table 2.3.2 on page 52) as a simple branching key.

Analysis

6 Look carefully at the four lizard images below. Identify characteristics that you could use to design an identification key for these four lizard families:

a Agamidae (dragons)
b Gekkonidae (geckos)
c Scincidae (skinks)
d Varanidae (monitors).



7 An ecologist sampled the earthworm population of two 75 m^2 areas by marking out three randomly placed 1 m × 1 m quadrats for each area. Each quadrat was sprinkled with formalin to bring the worms to the surface for counting. The number of worms recorded in each quadrat is given in the table below. Quadrats 1, 2 and 3 were in fallow (rested) pasture and quadrats 4, 5 and 6 were nearby in an area that had been overstocked with cattle.

Quadrat	Number of worms
1	46
2	54
3	41
4	9
5	13
6	15

a Estimate the size of the earthworm population in each 75 m^2 area.
b Infer one reason why the results are probably underestimates of the actual population sizes.
c Explore the conclusions that can be drawn about the difference in the size of the two populations.

8 As an arachnologist, you are often required to go into the field to conduct population surveys of spiders in different ecosystems. Your next field trip is into tall closed forest with an average canopy cover of 80%. Differentiate the various strata that you could divide the forest into for your quadrat sampling of spiders.

Reporting on an ecosystem

Use the process of stratified sampling to collect and analyse primary biotic and abiotic field data to classify an ecosystem.

Research and planning

Aim

- To complete a first-hand field study of a selected ecosystem
- To consider the biotic and abiotic factors that make up an ecosystem
- To estimate the abundance and distribution of selected organisms within an ecosystem and consider the factors that might affect them
- To consider some of the relationships between species within an ecosystem

Rationale (scientific background to the experiment)

Ecosystems are comprised of abiotic (non-living, environmental) and biotic (living) components. The interaction of abiotic and biotic factors can influence the energy and population dynamics of an ecosystem. Measuring these factors enables scientists to classify an ecosystem. This is important, as it enables scientists to begin to work towards effective ecosystem management.

To classify ecosystems, field guides and keys for the identification of plants and animals are useful. Ecologists must also take care to protect themselves from stinging or biting insects and spiders when observing particular parts of the ecosystem. For example, they may use a stick or pencil to gently lift the bark of trees or leaf litter. Personal protective equipment, such as gloves and boots, may also be useful.

When observing ecosystems, it is important to create the least possible disturbance. Ensure that you have permission from the appropriate authority if you have to collect specimens for analysis back in the school laboratory. Monitoring and analysis that can be completed in the field should be done there. Ensure rocks that have been upturned during the investigation are returned to their original position.

You need to be familiar with different kinds of aquatic and terrestrial ecosystems, so background reading is recommended. In this activity you will classify the ecosystem you study. You also need to be able to choose and conduct appropriate sampling techniques for estimating population sizes for plant and animal species.

Timing

60–90 mins

Materials

- pencils
- measuring tape (at least 10 m long)
- rope or cord
- 1 m × 1 m quadrat
- thermometer or temperature probe
- light meter
- anemometer (or devise a suitable scale, e.g. 5-point)
- pH meter or probe
- Secchi disc or turbidity tube
- oxygen meter or probe
- magnifying glass
- field guides (e.g. grasses, herbs, eucalypts, invertebrates)
- extra lined/unlined paper for notes and diagrams, if required

Risk assessment

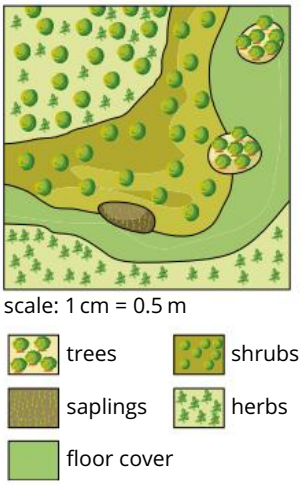
Assessment of risks includes chemical hazards and physical hazards. Before you commence this practical activity, you must conduct a risk assessment. Complete the template in your *Pearson Biology 12 Queensland Skills and Assessment* book or download it from your eBook.

Method

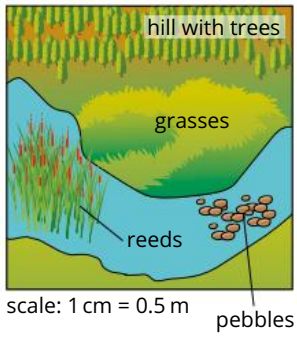
1. When you arrive at the chosen study site, take a few minutes to look around. Observe the main features, prominent landmarks, the kinds of vegetation in the area, dominant plant species and weather conditions.
2. Write a general description of the habitat, including abiotic and biotic features. Abiotic features will include geographical characteristics as well as local weather conditions. When describing biotic features, mention the type and density of vegetation. Consider features that will help you classify this ecosystem. For example, for terrestrial ecosystems, consider the amount of sunlight that penetrates the foliage of trees to reach the ground. For aquatic ecosystems, think about the salt or fresh nature of the water. Is the body of water a lake, stream, river or sea? Name the kind of ecosystem you are studying.

3 Make two diagrams to represent the area: a plan sketch and a profile sketch. Label your diagrams to indicate various features, or use a key. As a guide for plan sketches, refer to diagrams (a) terrestrial and (b) aquatic. For profile sketches of ecosystems, refer to (c) terrestrial and (d) aquatic.

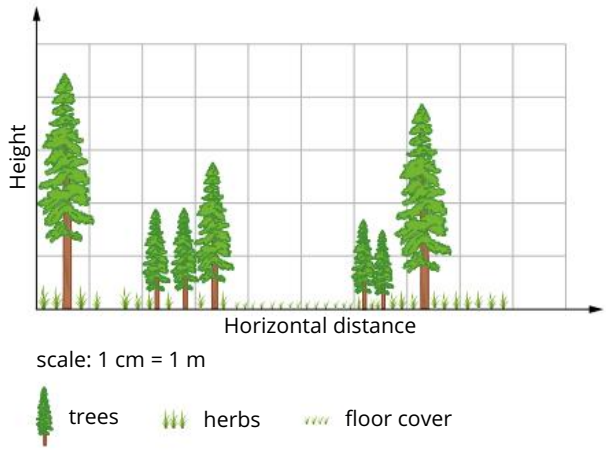
a



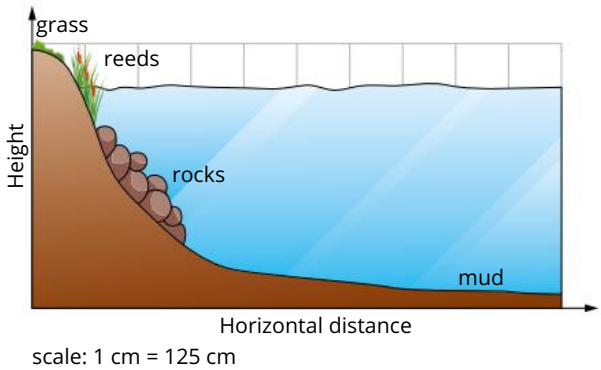
b



c



d



4 Use the plan and profile sketches to identify a transect line that runs through an area that varies over the distance. For example, the vegetation may change, taking in a body of water and undulations in the landscape. The transect line should be at least 10 m long.

5 Use the rope and measuring tape to mark the transect line in the ecosystem.

6 Draw a profile sketch of the ecosystem represented by the transect line (Figure 2.3.3 on page 48).

7 Walk the length of the transect line, observing and measuring as many abiotic factors as you can along the way. Record your observations in the table like the one on page 58.

8 Repeat your transect walk, recording the biotic factors you observe along the transect line. Record your data in a table like the one on page 58.

9 Identify the tallest plant species that occurs in the ecosystem.

10 Estimate the height of the tree. To estimate the height of extremely tall trees, follow the steps below.
- Fold a piece of square paper in half so that it forms a triangle.
- Hold the triangle in front of your eye. One short side should be parallel with the ground; the other short side should be parallel with the tree.
- Holding the triangle still, look along the long edge (the hypotenuse). Carefully move backwards until the top of the tree aligns with the hypotenuse.
- Mark your position on the ground and measure the distance from the base of the tree.
- Add your height to the distance measured from the base. This will give you the approximate height of the tree.

11 At regular intervals along the transect, estimate the canopy cover (Table 2.1.2 on page 23) of the tallest plant species.

12 Record your data in a table like the one on page 58.

Analysing

Raw data

1 Record your data about the abiotic factors in a table similar to the one below. Depending on the length of the transect line, the measurements may be taken at intervals greater than 1 m.

Summary of abiotic factors (some of these factors will not be present in your chosen ecosystem)

Abiotic factor	**Measurement along the transect line**										
	0 m	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m
air temperature • ground level • 1 m above ground level											
humidity											
soil temperature											
water temperature											
light intensity											
wind speed											
soil pH											
water pH											
water turbidity											
dissolved oxygen content of water											
flow rate of water (place an object in the water and measure distance covered over a given time, e.g. metres per minute)											

2 Record your observations about the biotic factors in a table similar to the one below.

Plant and animal species observed along the transect line

Plant species	**Animal species**

Canopy cover of the tallest plant species

Species:

Estimated height:

Transect position	**Canopy cover (%)**

Processed data

3 From the raw data, classify the ecosystem according to Specht's classification system.

Specht's structural classification of vegetation

Canopy cover of the tallest stratum					
life form of tallest stratum	70–100%	50–70%	30–50%	10–30%	<10%
trees >30 m	tall closed forest	tall forest	tall open forest	tall woodland	n/a
trees 10–30 m	closed forest	forest	open forest	woodland	open woodland
trees <10 m	low closed forest	low forest	low open forest	low woodland	low open woodland
shrubs >2 m	closed scrub	scrub	open scrub	tall shrubland	tall open shrubland
shrubs (S) 0.25–2 m	closed heathland	heathland	open heathland	shrubland	open shrubland
shrubs (NS) 0.25–2 m	n/a	n/a	low shrubland	low shrubland	low open shrubland
shrubs (S) <0.25 m	n/a	n/a	n/a	dwarf open heathland	dwarf open heathland
shrubs (NS) <0.25 m	n/a	n/a	n/a	dwarf shrubland	dwarf open shrubland
hummock grasses	n/a	n/a	n/a	hummock grassland	open hummock grassland
tussock grasses	closed grassland	grassland	grassland	open grassland	very open grassland
sedges	closed sedgeland	sedgeland	sedgeland	open sedge land	very open sedge land
herbs (forbs)	closed herbland	herbland	herbland	open herbland	very open herbland
ferns	closed fernland	fernland	fernland	n/a	n/a

S = sclerophyllous NS = non-sclerophyllous n/a = not applicable (does not occur naturally)

➤ Reflect and check that your data analysis demonstrates these characteristics

- ☐ Effective investigation of phenomena is demonstrated by the collection of sufficient and relevant raw data
- ☐ Accurate application of algorithms, visual and graphical representations of data is demonstrated by appropriate processing and presentation of data to aid the analysis and interpretation of data

Analysis

4 Explain the difference between biotic and abiotic factors.

5 Explain why flora is primarily used as the defining characteristic of a terrestrial ecosystem.

6 Explain why single abiotic measurements, such as air temperature, are not suitable to use for ecosystem classification.

7 Use some of your measurements of abiotic factors to classify the ecosystem you studied.

➤ Reflect and check that your analysis demonstrates these characteristics

- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough and appropriate error analysis
- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough identification of relevant trends, patterns and relationships
- ☐ Insightful and valid interpretation of evidence is demonstrated by drawing a valid and defensible conclusion based on the analysis

Interpreting and communicating

Conclusion

1 Summarise the features of an ecosystem used by Specht's classification system.

Improvements

2 If you were to repeat this experiment, identify the steps you would do differently. Consider how you:
   - **a** might change the methodology
   - **b** might improve your technique
   - **c** could reduce error and uncertainty.

Extension

3 There are other systems that can be used to classify an ecosystem. Research one of the following classification systems and reclassify the ecosystem you studied.
   - Holdridge life zone classification scheme
   - ANAE classification system
   - EUNIS habitat classification system

4 Research the mean annual precipitation and mean annual temperature for your ecosystem. Use these abiotic factors to characterise the ecosystem.

➤ Reflect and check that your evaluation demonstrates these characteristics

- ☐ Critical evaluation of processes is demonstrated by a discussion of the reliability and validity of the experimental process supported by evidence such as the quality of the data (as quantified in the error analysis)
- ☐ Critical evaluation of the conclusion is demonstrated by a discussion of the veracity of the conclusions with respect to the error analysis and limitations or sufficiency of the data
- ☐ Insightful evaluation of processes and conclusions is demonstrated by a suggestion of improvements or extensions to the experiment that are logically derived from the analysis of the evidence

Chapter review

02

KEY TERMS

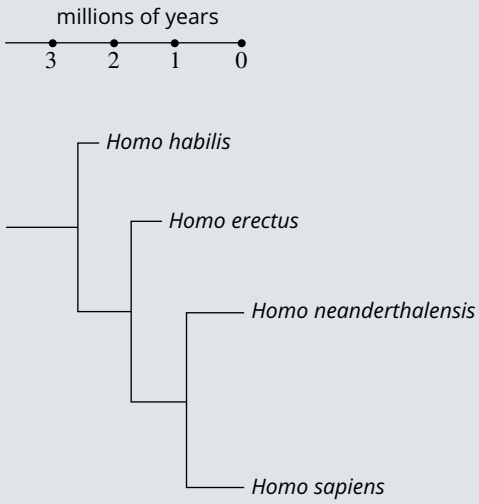
abiotic
bacteria
belt transect
binomial
binomial system
biological classification
biological species
bioregion
biotic
branch
calibration
clade
cladistics
cladogram
class
classification
competition
dichotomous key
disease
DNA hybridisation
domain
ecoregion
ecosystem
eukaryote
family
form
gene probe
genus
hierarchy
infectious
ingroup
interspecific hybrid
K-selection
K-strategist
kingdom
leaf
line transect
lineage
Linnaean system
Linnaeus
maximum parsimony
microhabitat
mitochondrial DNA (mtDNA)
monophyletic group
morphological
multi-access key
mutation rate
node
non-infectious
order
outgroup
paraphyletic group
pathogen
phylogenetic classification
phylogenetic tree
phylogeny
phylogram
phylum
polyphyletic group
polytomy
profile diagram
prokaryote
quadrat
r-selection
r-strategist
root
rooted tree
sister taxa
species
stratified sampling
subspecies
symbiotic relationship
taxon
taxonomy
transect
unrooted tree
variety
virus

KEY QUESTIONS

Retrieval

1 Identify which of the following statements best describes the phylogenetic tree shown below.

A scaled and rooted
B scaled and unrooted
C unscaled and rooted
D unscaled and unrooted



2 Select one of the advantages of using mitochondrial DNA over nuclear DNA for classification from the list below.

A Mitochondrial DNA is less susceptible to heat distortion.
B The rate of mutation in mitochondrial DNA is lower than in nuclear DNA.
C The rate of mutation in mitochondrial DNA is usually higher than in nuclear DNA.
D Mitochondrial DNA can only be inherited from the father of the offspring.

3 List the groups of the domain hierarchy from the largest (widest) group to the smallest (most defined) group.

Comprehension

4 Scientists sometimes change the way they classify certain organisms. Select the main reason for this.

A As scientists gain new information, their understanding of the relationship between organisms changes.

B Organisms are given different names in different countries; therefore, they will be classified differently.

C Some scientists think that different features are important and the classification depends on which ones are used.

D Organisms change from generation to generation; therefore, some will show characteristics that cause them to be classified differently from other members of the species.

5 Select the most appropriate description for the relationship between *Avicularia metallica* and *Poecilotheria metallica*.

A the same species with two names

B different subspecies of one species

C different species in the same genus

D different species in different genera

6 Explain the classification of organisms according to:

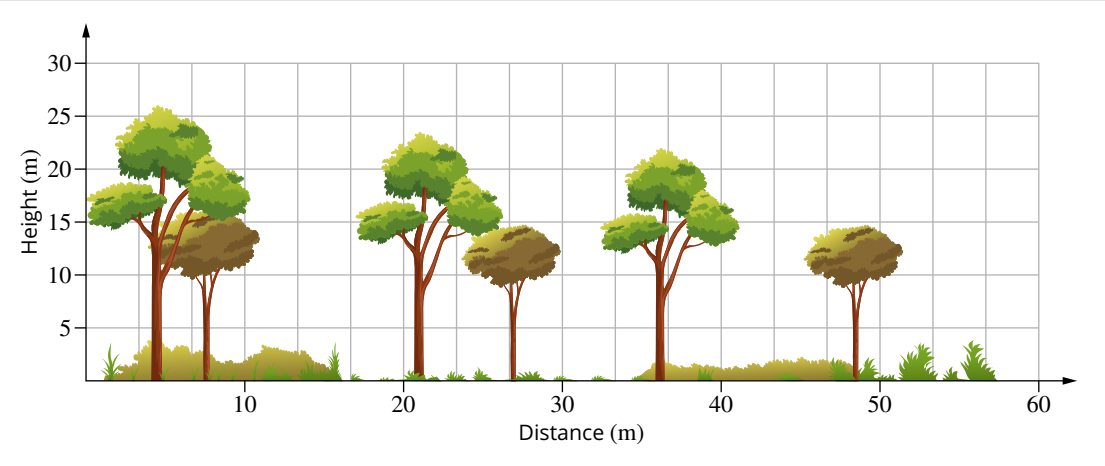
a predation

b competition

c symbiosis

d disease

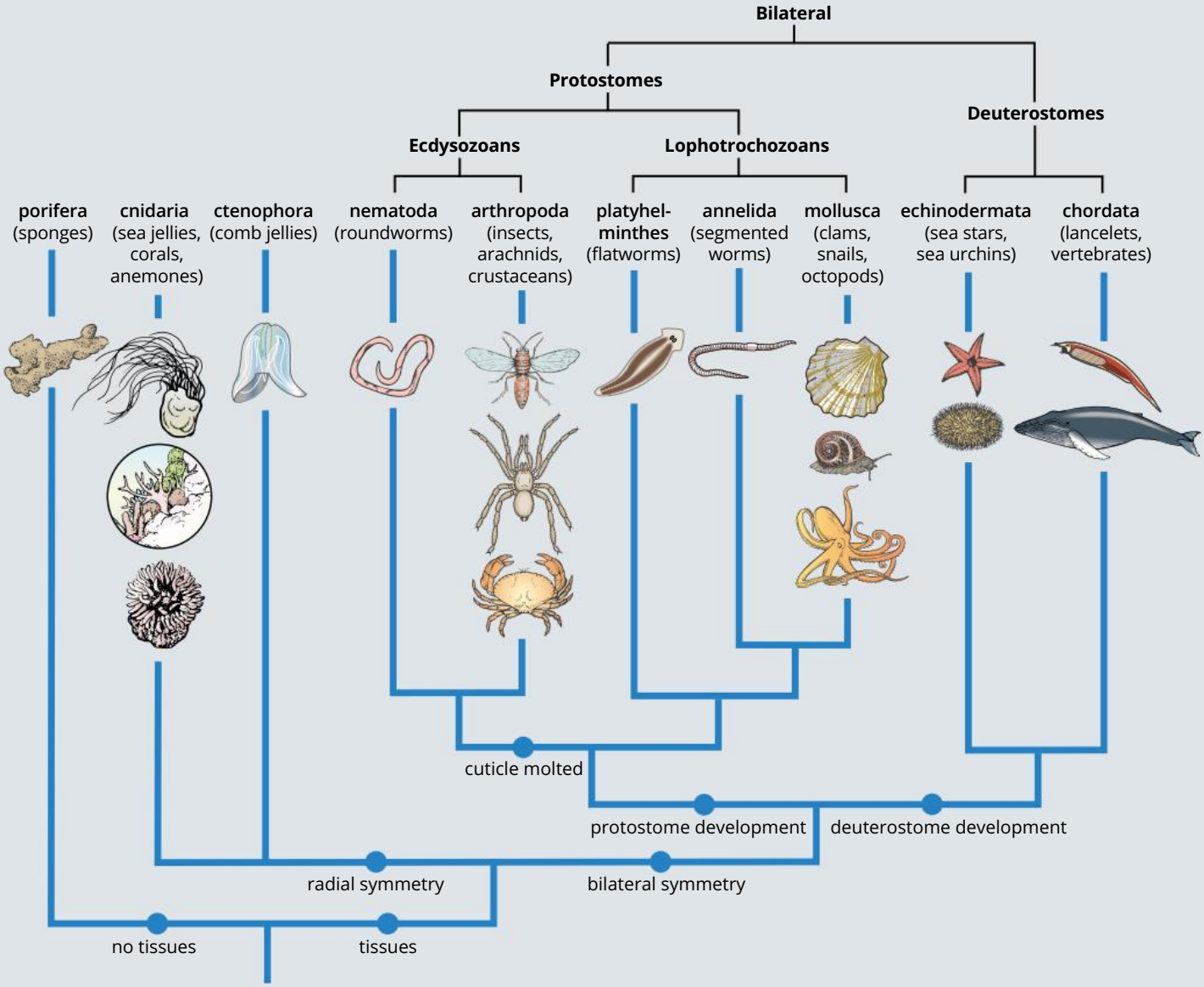
7 Use Specht's classification (Table 2.1.2 on page 23) to classify the habitat shown in the transect profile below.



Analysis

8 A student was interested in the relationships of the following groups of marine invertebrates: sponges and corals, molluscs and annelids, echinoderms and crustaceans, and roundworms and segmented worms. Examine the phylogenetic tree below and determine which of the four groupings of animals of interest are the most closely related.

An evolutionary tree of some major animal phyla



9 Contrast the three-domain model of classification with the five-kingdom model.

10 The separation temperature of DNA from a single species is 86°C. A DNA hybridisation experiment was performed between three species (A, B and C). The results of the experiment are shown in the table below.

Separation temperature of DNA

DNA mix	Separation temperature (°C)
A–B	78
A–C	72
B–C	81

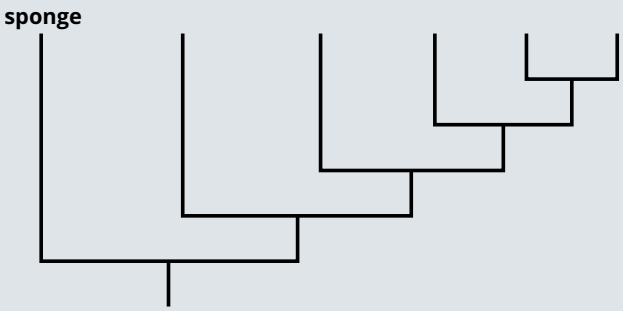
Draw a cladogram that would be most likely generated from this information.

11 The following table is a character matrix that lists six animals and four morphological characteristics. A tick (✓) indicates that the animal has this characteristic while a cross (✗) indicates that the characteristic is absent.

Morphological characteristics of selected species

Animal	Spinal cord (vertebra)	Legs	Hair	Placenta present during pregnancy
blue-tongue lizard	✓	✓	✗	✗
platypus	✓	✓	✓	✗
red fox	✓	✓	✓	✓
sponge	✗	✗	✗	✗
funnel-web spider	✗	✓	✓	✗
red kangaroo	✓	✓	✓	✓

Based on the features above, complete the phylogenetic tree below.



12 In the past, phylogenetic trees could only be generated using morphology. Modern taxonomists tend to rely on scoring the differences in DNA between two species in order to determine how closely related they are. Scientists were studying the relationships between a number of species in the genus *Conus*. This is a genus in the phylum Mollusca. All members of the genus have cone-shaped shells.

Cytochrome *c* oxidase I (COI) is an enzyme found in many species, including the four species of *Conus* below. The table shows the number of nucleotide differences between pairs of the species.

Nucleotide differences in cytochrome *c* of selected species of *Conus*

Species	*C. marmoreus* (M)	*C. chaldeus* (C)	*C. omaria* (O)	*C. magnus* (MG)
C. marmoreus (M)		8	35	9
C. chaldeus (C)			36	10
C. omaria (O)				41

a Create a possible cladogram for these species using the data obtained from the DNA sequences.

b A DNA hybridisation experiment was performed between the four members of *Conus* using the *COI* gene. Predict which pair of species would have the lowest separation temperature and explain the prediction.

13 The table below has descriptions of six birds from the mid-eastern region of Australia. Create a dichotomous key to classify these six species, using these descriptions.

Characteristics of six bird species

Common name	Scientific name	Appearance, behaviour and habitat	Egg
brown quail	*Coturnix ypsilophora*	Small, round, ground-dwelling, with brown or rufous speckled appearance, short grey beak and red eyes. When startled, will explode into flight and then dive headfirst into cover. Lives in dense shrub and tall grass.	• speckled brown • 28 mm × 22 mm • ground nest
magpie goose	*Anseranas semipalmata*	Black and white pied goose with distinctive knob on crown, black head and neck, wing tips and short-rounded tail, white body with orange legs with partially webbed feet. Pink facial skin and yellow orange bill. Black eyes. Lives in wetlands and marshy areas.	• stained brown • 74 mm × 50 mm • platform nest in wetlands
emerald dove	*Chalcophaps indica*	Small dove-sized bird with pinkish/violet-tinted brown head and body. Underside of wings chestnut brown. Top of wings iridescent emerald green. Tail dark brown with black band. Pink feet and orange to red beak. Lives in rainforest or dense vegetation along creek banks.	• whitish • 29 mm × 22 mm • nest in trees or in staghorns
scrub turkey	*Alectura lathami*	Small turkey-sized, black-feathered rainforest bird. Bright red head and neck with yellow wattles. Yellow eyes and black to yellow beak. Long toes on feet used for raking leaf litter into large mounds.	• whitish • 92 mm × 63 mm • laid in large mound
brolga	*Grus antigone*	Large grey crane, with a featherless red head and grey crown. The legs are grey and there is a black dewlap under the chin. Females are shorter than males. The energetic dance performed by the brolga is a spectacular sight. Inhabits large open wetlands and grassy plains.	• white blotched with brown and purple • 95 mm × 61 mm • platform nest in wetlands

14 As a biologist, you have been requested to undertake population density transects in the tidal rock pools shown below. Distinguish the various regions of the rock pool that you could use for strata sampling.

15 A 10 m transect was conducted from a small creek bank and up a 2 m high slope.

a Create a profile diagram of the transect line, using the transect data below.

b Identify any problems or areas of concern based on the data presented and the profile you have generated. State any inferences you have made in your analysis of the data.

Transect data

Transect mark (m)	Vegetation type	Canopy cover (%)	Substrate type	Slope (%) (rise/run × 100)	Soil pH	Air temp. (°C)	Soil temp. (°C)	Notes
0	bulrushes and reeds	• grass 95 • bare soil 5	black mud	1	6	22	20	samples T1.0.1 *Typha* sp., T1.0.2 *Juncus* sp.
1	tussocky wiry grass	• grass 90 • bare soil 10	wet clay soil	1	6.2	22	21	sample T1.0.3 grass
2	tussocky wiry grass	• grass 90 • bare soil 10	clay soil	2	6.2	22	22	
3	tussocky wiry grass	• grass 80 • herb 5 • bare soil 15	clay soil	2	6.2	22	22	samples T1.0.4 grass, T1.0.5 herb
4	stunted tussocky wiry grass	• grass 50 • bare soil 50	clay stony soil	5	5.5	24	24	samples T1.0.6 grass, evidence of orange substance oozing down slope
5	none	• bare soil 100	bare rock and soil	5–0	4.8	24	24	more orange substance oozing down slope, slight smell rotten egg gas, start of horizontal ledge, collected sample of slime
6	none	• bare soil 100	bare rock and soil	0	3.9	24	26	layer or orange slime material on surface
7	small tussocky grass	• grass 80 • bare soil 20	clay loam	0–5	6.3	24	24	orange slime no longer present, vegetation present again, sample T1.0.7 grass, slope resumes
8	tussocky wiry grass	• grass 95 • bare soil 5	clay soil	0–5	6.6	25	24	grass health, slope eases off to horizontal section again
9	low shrubs 0.5 m and grass	• shrub 75 • grass 10 • bare soil 15	clay loam	0–5	6.9	26	23	photo 15 shrub flower and leaf/whole plant, slope resumes
10	shrubs 0.5 –1 m and grass	• shrub 80 • grass 15 • bare soil 5	clay loam	5	6.9	26	23	shrubs increasing in height

Knowledge utilisation

16 Using the Queensland Government's 'Search regional ecosystem descriptions' website, draw a flow chart representing the three steps required to find the RE ID (regional ecosystem identification number) for a research site based on the Mitchell grass downs with cracking clay plains and two dominant grass species, *Astrebla pectinate* and *Iseilema* spp.

17 In early December 2016, violent storms swept through Mt Warning National Park in northern New South Wales. Strong winds, driving rain and lightning strikes resulted in many ancient trees being thrown down, exposing large swathes of rainforest that had previously been shut off from direct sunlight.

- **a** Design a systematic way of collecting data from the recovering rainforest. The methods must include the recording of succession species (plants that initially take advantage of the space created in the forest, but may one day be replaced by slower growing, larger plants), the impact on animal species and population densities, identification of all species present and an assessment of what species have been lost.
- **b** Decide what data you would need to see over time to allow you to judge whether or not the rainforest will regenerate or whether it will remain a weed-infested area.

CHAPTER 03

Biodiversity

Biodiversity is short for biological diversity. Often, biodiversity refers to the variety of organisms living within an ecosystem. Biodiversity is important for the well-being of ecosystems, as high levels of biodiversity help to stabilise ecosystems. In other words, high levels of biodiversity enable ecosystems to better cope with sudden changes. Ecologists analyse levels of biodiversity within ecosystems in order to measure an ecosystem's health, and to consider human practices that may be harming, or helpful in maintaining, biodiversity.

By the end of this chapter, you will understand the nature and importance of biodiversity and some of the factors, both biotic and abiotic, that influence it. You will understand how species interact with each other to influence the biodiversity of an ecosystem. You will have discovered how abiotic factors, such as soil structure and composition, determine the vegetation cover of an area and how this influences other species. You will have learnt about how biodiversity is measured and how it changes over time and space. You will learn to apply measures, such as Simpson's diversity index, and how to measure species richness and abundance. You will also have learnt how to measure a number of abiotic factors that influence the biodiversity of any area.

Syllabus subject matter

Topic 1 • Describing biodiversity

BIODIVERSITY

- recognise that biodiversity includes the diversity of species and ecosystems
- explain how environmental factors limit the distribution and abundance of species in an ecosystem
- determine diversity of species using measures such as species richness, evenness (relative species abundance), percentage cover, percentage frequency and Simpson's diversity index
- use species diversity indices, species interactions (predation, competition, symbiosis, disease) and abiotic factors (climate, substrate, size/depth of area) to compare ecosystems across spatial and temporal scales.

SCIENCE AS A HUMAN ENDEAVOUR

International biodiversity protection: International agreements about biodiversity protection, such as the World Heritage Convention, are based on the premise that local, regional and international biodiversity represent a global resource, vital for human survival, that should be maintained for future generations.

MANDATORY PRACTICAL 2

Determine species diversity of a group of organisms based on a given index.

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3.1 Biodiversity and its measurement

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recognise that ecosystems are diverse at a number of different levels
- calculate percentage cover
- calculate percentage frequency
- calculate species richness
- calculate species evenness
- apply Simpson's diversity index
- understand and describe species interactions
- evaluate the influence of species interactions on species richness, abundance and distribution.

Identification and measurement of biodiversity in Australia is important for both conservation and economic reasons. Considerable time and money is expended in order to catalogue and monitor ecosystems across the country. This module will focus on the types of ecosystems present in Australia, the various ways ecologists can calculate the level of biodiversity present in the ecosystems, and the different ways biodiversity can be described and characterised. It will also outline factors that influence the biodiversity of ecosystems, such as species interactions.

TYPES OF BIODIVERSITY

Biodiversity encompasses all of the species present in the world, all of the ecosystems to which those species belong and all of the interactions between those species. It includes taxonomic, genetic and ecological variation. The maintenance of biodiversity is critically important because diverse ecosystems are more stable than uniform ecosystems. Diverse ecosystems are more likely to withstand and survive sudden changes than less diverse ecosystems. Diverse ecosystems also provide many of the needs of human populations.

Biodiversity can be measured at three different levels: genetic, species and ecosystem diversity.

Genetic diversity

Genetic diversity refers to the number of different alleles possessed by a species. Genetic diversity within a species is determined by DNA techniques such as DNA profiling and DNA sequencing. Populations of species with greater genetic diversity are more likely to tolerate changes in environment. Less variable populations face a greater possibility of extinction. For example, cats show considerable genetic diversity. This is shown by the variety of characteristics they display (Figure 3.1.1).

FIGURE 3.1.1 Cats display a broad variety of different traits, all controlled by different genetic variants.

Species diversity

Species diversity is the number of different species present in a particular ecosystem. Ecosystems with more species, such as the Amazon jungle (shown in Figure 3.1.3a), tend to be more robust and are less likely to collapse as a result of small changes in environmental conditions or species constituency.

Ecosystem diversity

Ecosystem diversity across the world considers the various biomes and the variation within those biomes. For example, within the rainforest biome many different habitats can be found: rivers, lakes, clearings, understoreys, canopies and hollow trunks of dead and fallen trees. The more habitats and microhabitats there are within an ecosystem, the more species are likely to be present. This is because each species has a set of environmental conditions within which suitable conditions are required for their survival (their **tolerance range**), and a greater number of different sets of conditions means a greater number of species will be catered for.

The Earth contains a vast array of different ecosystems. Each of these ecosystems has its own unique traits. They have different abiotic factors and these influence the types of species that are present and how these species interact with each other.

The Earth's surface is approximately 29% terrestrial and 71% aquatic. Of the aquatic area, around 97% is marine and the rest is divided between all freshwater types: lakes, rivers and aquifers (Figure 3.1.2). A large proportion of the Earth's fresh water, about 69%, is held in glaciers and the polar ice caps.

The temperature and rainfall at the equator is very different to that of the poles. The extreme cold and lack of liquid fresh water at the poles means that plants do not grow there. On the other hand, the forests of the Amazon, which straddle the equator, contain an enormous diversity of life, supported by high rainfall, warm temperatures and consequent fast decay of organic material. Comparison between the Antarctic and the Amazon demonstrates the difference that abiotic factors make. There are few species in the Antarctic and no amphibians or reptiles, but there are so many species present in the Amazon that many of them have not even been identified (Figure 3.1.3).

A biome is a group of ecosystems which have similar climate and other conditions, and which extend over a large area.

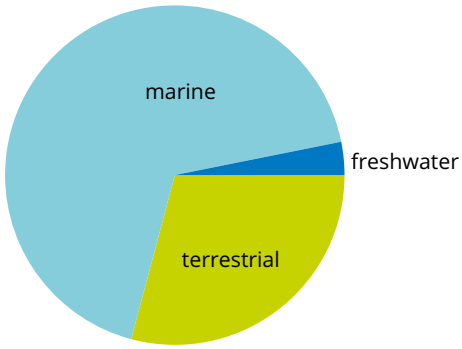


FIGURE 3.1.2 The division of the Earth's surface.

FIGURE 3.1.3 (a) The jungles of the Amazon have a huge number of different species but there are relatively few species in (b) Antarctica.

Table 3.1.1 shows a range of terrestrial biomes and some of their typical biotic and abiotic characteristics. Some biomes cover large areas of the world and even though each biome will have similar conditions, variations will occur. Table 3.1.1 gives an overview of the main terrestrial biomes, but individual sites may vary from the average.

TABLE 3.1.1 An overview of terrestrial biomes

Biome	Position	Approximate temperature range	Precipitation	Soils	Vegetation	Animals	Biodiversity
tundra	• 60–75°N • sub-Antarctic islands such as Macquarie Island • alpine regions may also have tundra with altitudes being higher closer to the equator	• –70–13°C	• 255–500 mm yearly, mostly as snow, with higher precipitation in coastal areas	• thin and nutrient-poor • subsoils remain permanently frozen	• grasses, mosses, lichens, small shrubs	• birds, mammals, insects • few reptiles or amphibians • many species migrate away during winter	• low • higher in summer than winter
taiga and boreal forest	• 65–50°N	• –54–21°C	• 300–850 mm yearly, mainly as summer rain	• generally acidic and low in nutrients	• evergreen trees such as conifers • lichens and mosses also present	• typically, few species except for insects • Canadian boreal forest has around 32 000 species of insect • lacks many earthworms, millipedes and other similar species that consume leaf litter thus slowing leaf litter breakdown	• low • higher in summer than in winter
tropical rainforest	• 23.5°N – 23.5°S	• 25–35°C	• 1520–10 200 mm per year	• generally shallow and nutrient-poor • high rainfall causes leaching of nutrients	• dense vegetation with tall trees and an equally dense understorey • little vegetation on the forest floor due to lack of light • Darwin stringybark (*Eucalyptus tetrodonta*)	• many species of most phyla	• high
temperate rainforest	• approximately 25–50°N and 25–50°S	• 0–27°C	• 3500 mm per year	• deep, loamy and rich in nutrients	• tall trees, dense understorey • ferns are common	• most phyla present • fewer mammals than the tropical rainforest	• high

Table 3.1.1 continued

Biome	Position	Approximate temperature range	Precipitation	Soils	Vegetation	Animals	Biodiversity
desert	• various latitudes but mostly inland in continents	• –18–50°C	• less than 500 mm annually	• coarse textured, generally shallow • nutrient-poor with poor water-holding ability	• vegetation sparse or absent • most plants are able to store large amounts of water (succulent) • in Australia, mallee eucalypts and acacias may be present	• insects, mammals, reptiles, birds all present but most are nocturnal	• low
grasslands and savannas	• savannas are mostly between 23.5°N and 23.5°S • grasslands are mostly between 23.5°N and 54°N and 23.5°S and 54°S	• variable depending on latitude	• up to 1020 mm annually but very variable depending on latitude	• deep, loamy and rich in nutrients in temperate zones • in tropical zones, soils are thinner with less decaying organic material present	• mostly grasses with small shrubs • savannas contain sparsely separated trees	• most phyla are represented • many large mammals; most of the world's hoofed mammals are found in grasslands	• high
alpine	• various latitudes at altitudes above 3000 m to the snow line • alpine regions start at lower altitudes further from the equator	• cool to cold, colder at higher altitudes • –10–18°C	• around 300 mm per year, mostly as snow	• similar to the soils of tundra but variable depending on altitude and slope	• variable ranging between evergreens, shrubs and grasses	• insects, mammals and birds present but few, if any, reptiles	• generally low but higher in summer than winter

Some of the most diverse ecosystems in the world are not terrestrial; they are the ecosystems of the coastal marine environment. Reefs, rocky platforms, sandy beaches and estuaries are all sites of coastal ecosystems. All of these ecosystems have some common characteristics but they also have their own unique biotic and abiotic factors.

Coral reefs, such as the Great Barrier Reef, are large, complex and diverse ecosystems. For example, the Great Barrier Reef is home to around 2100 different species of animals, along with plants, protists and countless species of bacteria. Some areas have deep water, some are quite shallow and the reef meets the land along the coast of mainland Australia and along many coral islands (Figure 3.1.4).

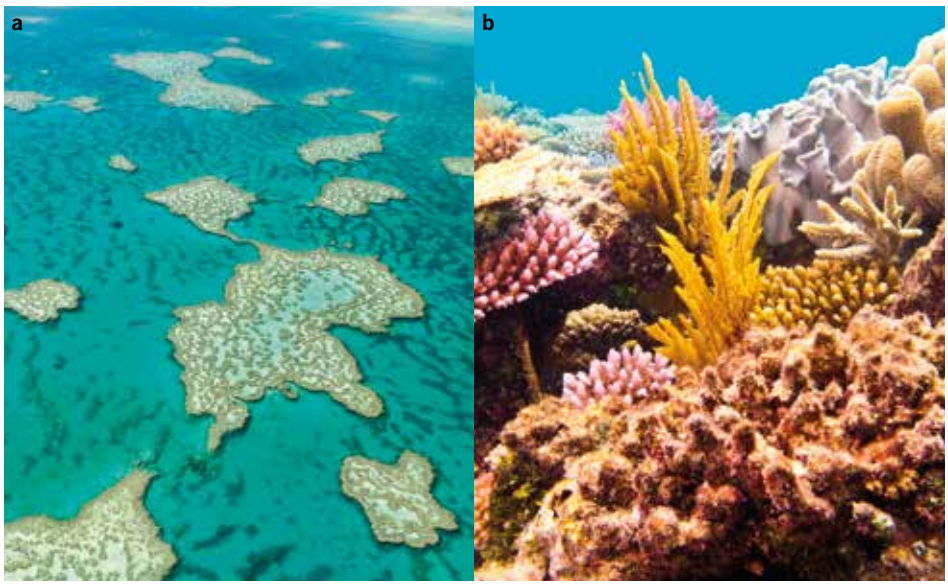


FIGURE 3.1.4 (a) The Great Barrier Reef extends over a considerable area. (b) The reefs contain many diverse species.

FIGURE 3.1.5 The intertidal zone is exposed to the air for parts of the day. This requires organisms such as sea stars to have features that allow them to live in both air and water.

The **littoral zone** is the region where the ocean meets the land. It extends from the high-tide mark into the sea to the depth beyond which light does not penetrate well enough for photosynthetic organisms to grow attached to the sea floor. Each of the sub-zones within the littoral zone has its own challenges for living things and its own suite of organisms. The **intertidal zone** is a particularly challenging environment. It is the part of the littoral zone that is sometimes exposed to air and sometimes under water. Organisms of the intertidal zone must cope with very variable conditions (Figure 3.1.5).

Estuaries form where rivers flow into the sea. The water gradually becomes more and more saline. Water that is neither fully saline (as seawater) nor fully fresh is called **brackish**. Among the organisms that cope well with brackish environments are mangroves, which is why mangrove swamps are common in estuaries in tropical and subtropical regions. Mangroves also cope well with the alternate drying and flooding of the intertidal zone (Figure 3.1.6). Mangroves form large forests along the intertidal zones that provide support for many animals. They provide cover for small fish and many invertebrates and their larvae, which protects the animals from predators.

FIGURE 3.1.6 Mangroves along the intertidal zone have their roots submerged for part of the day.

Producers are organisms that make their own food. Consumers are organisms that get the organic compounds they need by consuming other organisms or their products.

Mangroves, sea grass and kelp forests are all found in coastal environments. They are a rich source of marine biodiversity because not only are they **producers**, supplying a rich food source for the **consumers** in the ecosystem around them, they also provide a secure habitat for many species. Mangroves and other sea forests provide nurseries for many fish species.

Australia is very diverse. It features most terrestrial biomes (except tundra and taiga) and a large variety of freshwater and different marine habitats are represented somewhere across the continent, its surrounding coastline and oceans.

MEASURING BIODIVERSITY

Ultimately, ecosystem biodiversity relies upon the diversity of species present. The diversity of species able to inhabit an ecosystem is determined by the genetic composition of the organisms, and by the interactions of those organisms with other species present and the abiotic environment.

Determining the level of biodiversity in a particular area helps to inform decisions about how the area should be treated. Some areas, like a tropical rainforest, are highly diverse and others, like a field of wheat, have little diversity.

A number of measures are used to calculate the diversity of specific areas. Each of these measures relies on sampling in order to make estimates. Sampling is necessary because counting every individual of any particular species within an ecosystem is normally impossible because of time, money and the practicalities of finding and identifying each individual.

Estimates derived through sampling are only as good as the samples used. Sample sites should be decided by unbiased means and sample sizes should be as large as possible (Module 2.3). Measuring biodiversity involves identifying and measuring a range of different factors that give a picture of how many species are present in a region and how abundant they are. Every different region has its own biodiversity, including your backyard. The measures of biodiversity can be applied in the same way to an artificial environment, such as the pasture in Figure 3.1.7, or to a rainforest that has existed for hundreds or thousands of years.

FIGURE 3.1.7 A section of pasture which, despite seeming to be uniform, has its own biodiversity that can be measured.

Percentage cover

When measuring the number of trees in a small area, it is relatively easy to count the number of stems and come to an accurate count, but the same is not true when measuring organisms such as grass plants. To count each individual stem of grass in even a small forest would be impossible. Another measure of abundance is needed. One way to develop an estimate of the abundance of organisms is **percentage cover**. Percentage cover is a measurement of the proportion of an area covered by an organism.

Percentage cover is a useful measure of the relative abundance of plant species, as it gives a fair measure of each species contribution and influence in an area. Due to its size, a large tree has a much greater influence on other species in the area than a small bush. The tree provides more food for herbivores and a greater number of nesting sites. There is a greater range of microhabitats in a large tree, so it supports more species. A tree also has a greater influence on its surroundings than a small bush because its larger size means it consumes more resources such as water and soil nutrients and will therefore limit what other plants can live nearby.

In order to obtain an accurate measure of the relative abundance and the influence of species using percentage cover, it is necessary to use multiple sample plots (Module 2.3). Because plants are often seasonal in their abundance, the same plots should be surveyed several times during the year and ideally over several years.

There are four ways to measure percentage cover of plants:

- **basal cover**, which is a measurement of the cross-sectional area of the stem or stems at soil level
- **ground cover**, which is the area of the soil covered by plants, plant litter, rocks or any other material
- **leaf cover**, which measures canopy coverage and can be determined by the area of shadow cast by the canopy when the sun is directly above the sample site
- **canopy cover**, which measures the total area covered by the plant.

Leaf cover and canopy cover are similar. However, when estimating leaf cover, gaps in the middle of the plant where a shadow would not be cast are not included in the count. These areas are included when estimating canopy cover. In practice, these two measures generally give very similar results because plants tend to spread their leaves for greatest light capture. Most trees allow very little light to reach the ground, even though their foliage may seem sparse. Shadows cast by plants tend to have very few gaps when the plant is covered in leaves.

Evergreen plants possess leaves all year around. Deciduous plants lose their leaves, usually during autumn.

Evergreen plants, such as the eucalypts in Figure 3.1.8, seem to have very different densities of foliage but the shadows cast by both of the foreground trees do not have many breaks. Deciduous plants, on the other hand, may give very different values depending on the season. The area of the canopy will not vary much, as the branches remain through the winter, but foliage cover would be much lower in winter as the loss of leaves produces gaps that allow light to pass through to the ground.

FIGURE 3.1.8 Shadows cast by different trees show that, despite what appear to be differences in density of foliage, there are few gaps in the shadows. The leaves are spread to capture the maximum amount of light.

Quadrats are small sections of the area to be surveyed, chosen by an unbiased method. They may be any shape but must be uniform in both size and shape. The larger the number of quadrats sampled, the more accurate estimates are likely to be.

Calculations of both total leaf cover and total foliage cover within a quadrat can result in values greater than 100% since there can be an overlap between different individuals within the one quadrat (Module 3.2). The dense rainforest shown in Figure 3.1.9 is an example of this. There are different layers of foliage, which overlap each other. Tree trunks can also be seen, and the tree canopy would add another layer to the quadrat.

FIGURE 3.1.9 Total leaf cover and total foliage cover of a quadrat of this rainforest would have a value greater than 100% because there is overlapping foliage.

Quadrat maps are generally drawn from an aerial view. Figure 3.1.10 shows a quadrat of a small patch of daffodils growing in a field of grass and clover. The daffodils are taller than the clover and grass.

Percentage cover can be used in conjunction with aerial or satellite surveys showing vegetation cover. Satellite images taken at different times in the same year, and in different years, can be used to calculate total percentage cover of the Earth's vegetation (Figure 3.1.11). This allows scientists to identify changes over time. Using satellite-mounted monitors, visible and infrared light reflection is monitored from space and the results are used to calculate the amount of green vegetation. The result is the Vegetation Index. Averages are then calculated to create the Normalized Vegetation Index.

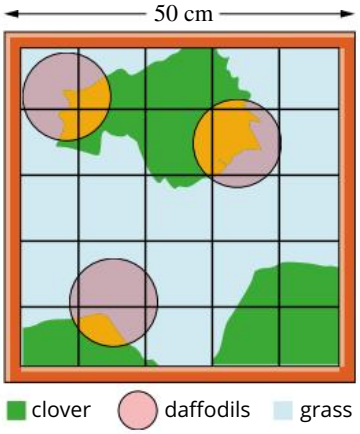


FIGURE 3.1.10 A 50 cm × 50 cm quadrat of grass and clover containing three daffodils. Daffodils overshadow the grass and clover. A calculation of percentage cover could exceed 100%.

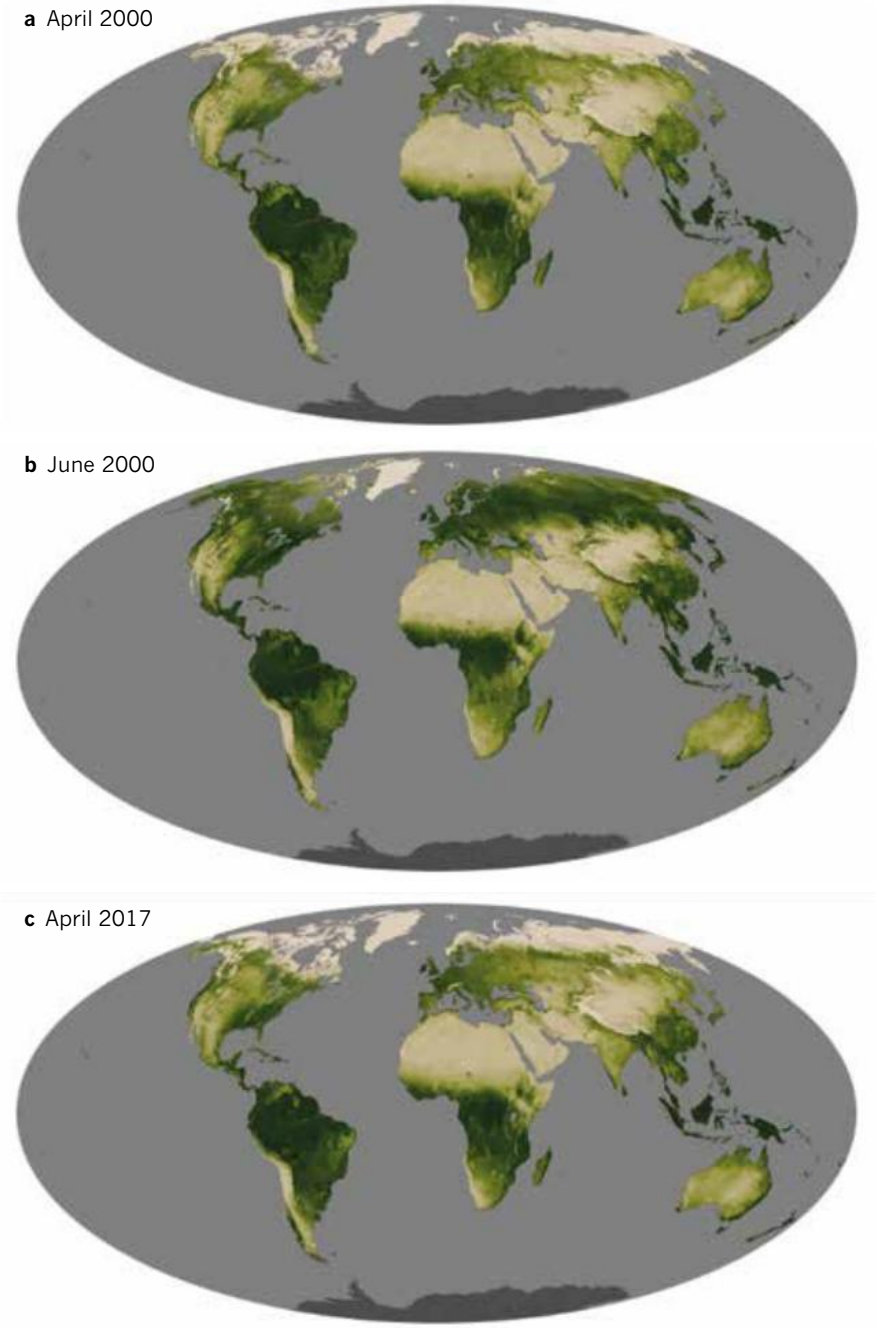


FIGURE 3.1.11 Satellite images taken by NASA show the Earth's vegetation coverage measured by level of greenness is different between (a) April 2000 and (b) June 2000, and between April 2000 and (c) April 2017.

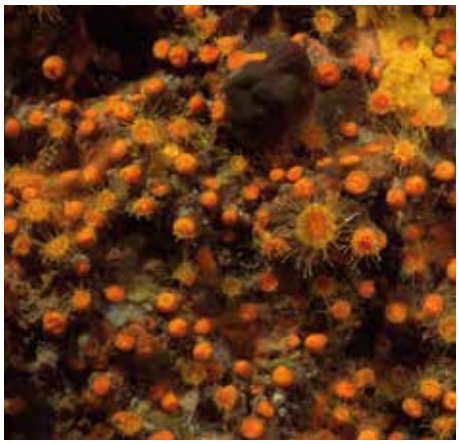


FIGURE 3.1.12 Percentage cover can also be used to measure the relative abundance of animal species, like these corals.

Percentage cover is generally used for estimating the contribution of plants to a particular area, although it can also be used for other organisms such as corals (animals), seaweeds (protists) and other organisms that are fixed in one place (Figure 3.1.12). While percentage cover can be used on a large scale, such as estimating forest coverage with aerial or satellite imaging, it is usually used in sample quadrats.

Calculating percentage cover

Percentage cover can be calculated by estimating the percentage of each quadrat covered by any particular species, but this is quite subjective and prone to error. A more accurate method is to divide the quadrat up into smaller units.

For example, if the quadrat is 50 cm × 50 cm, divide it up into smaller squares of 10 cm × 10 cm and represent these on a piece of graph paper. Each large square on the graph paper represents 4% of the total area, and small squares represent 0.04% of the total area. As shown in Figure 3.1.13, draw the coverage of the plants on the graph paper to allow for better estimation of percentage cover and to create a permanent record. Photographs may also be used, but a scale or grid should be included in the photograph to assist in estimating size. Remember to create a key for the different species present.

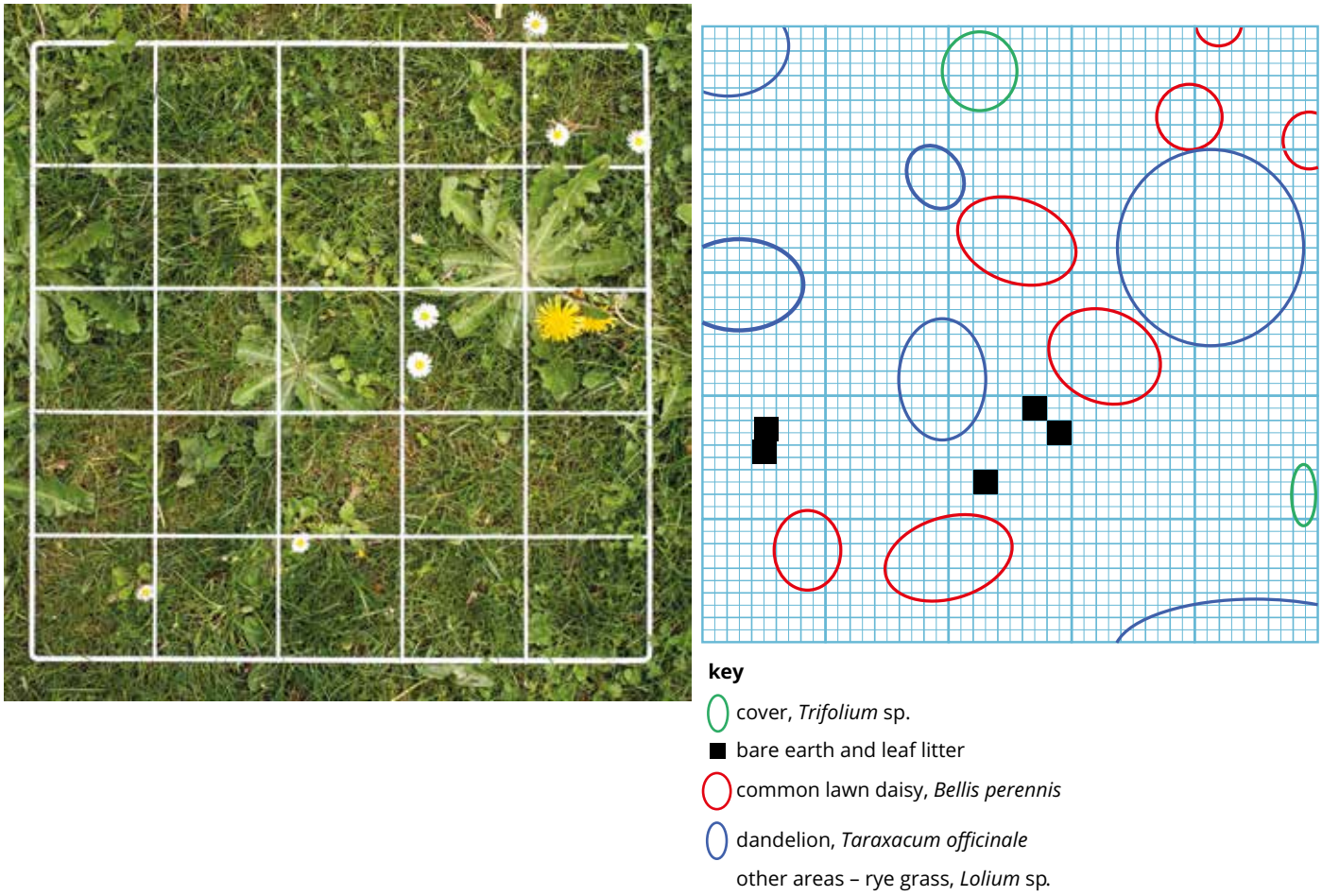


FIGURE 3.1.13 Pasture species in a 50 cm x 50 cm quadrat plotted on a piece of graph paper.

Before the survey begins, an agreement must be reached on how to count squares that are only partially covered. For the example shown in Table 3.1.2, based on Figure 3.1.13, squares were only counted if coverage was more than 50%. Plants sometimes overlap each other, so percentage cover may exceed 100%.

TABLE 3.1.2 Calculation of percentage cover

Type of cover or species	Tally of small squares covered by each individual of the species	Total squares covered by the species	Percentage cover
Trifolium sp.	23, 8	31	0.04 × 31 = 1.24%
Lolium sp.		1980	0.04 × 1980 = 79.2%
Bellis perennis	6, 11, 12, 26, 30, 33, 15	133	0.04 × 133 = 5.32%
Taraxacum officinale	32, 40, 12, 55, 183, 34	356	0.04 × 356 = 14.2%
bare earth and leaf litter		20	0.04 × 20 = 0.8%
total			100.76%

Agreement on the type of coverage to be used must be determined before beginning the study.

In the example given, the metric being used is ground cover, due to the type of plants surveyed. If a different type of survey area was to be considered, then one of the other metrics—basal, leaf or canopy cover—could be used. Plotting would be performed in a similar way but the results would be different.

Percentage cover is very useful for estimating abundances of herbaceous plants, which are difficult to count, but it can result in overestimates of the abundance of large plants such as trees. Their size means they cover large areas, but there may be very few individuals.

Averaging the percentage coverage of each species over a large number of quadrats gives a good estimate of the abundance of each particular species in the survey area. Poor positioning of quadrats within the study area can lead to rare species being missed. Quadrats within a sample region should be randomly generated and sufficient in number to ensure that all parts of the region are sampled.

Percentage frequency

Calculating the abundance of plants across large areas can be quite challenging, so another measure often used by field biologists is **percentage frequency**. Percentage frequency is a measure of the appearance of plant species within sample quadrats. Quadrats within the area are selected randomly and each quadrat containing the species of interest is scored. The percentage frequency is the number of quadrats containing the species of interest divided by the number of quadrats sampled, expressed as a percentage.

Calculating percentage frequency

In Figure 3.1.14, the entire area was divided up and 10 quadrats were chosen at random (marked with a yellow star). The plant was observed in 1 of the 10 chosen quadrats, so the percentage frequency was calculated to be 10%.

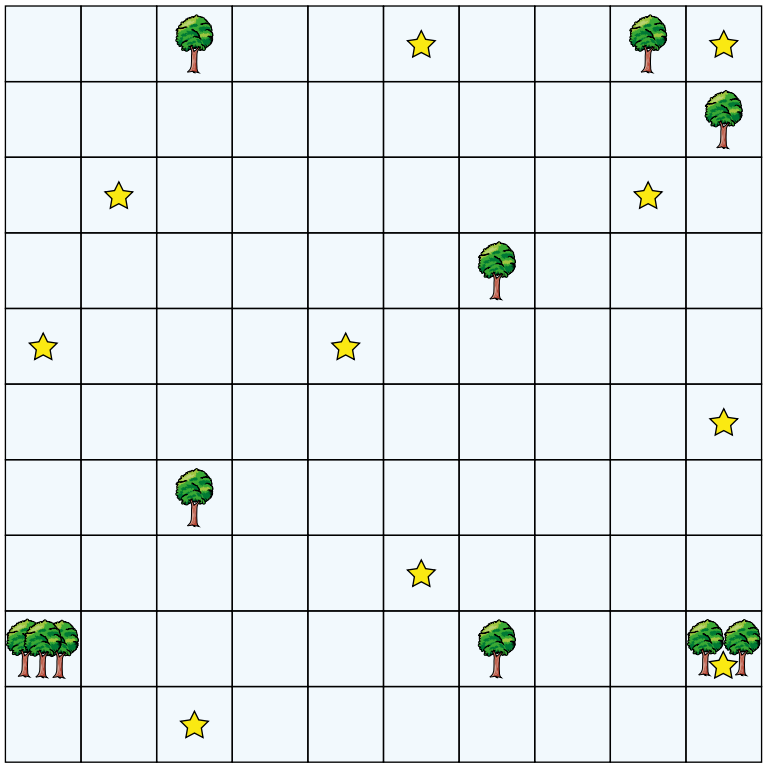


FIGURE 3.1.14 The percentage frequency of this area was measured using sample quadrats and calculated as 10%.

Percentage frequency can also be used for calculating the diversity of a large area, such as a forest. Quadrats are assigned a number or letter and their position indicated on a map of the survey area. Each species in each quadrat is identified and the presence or absence in individual quadrats is recorded in a table (Table 3.1.3).

TABLE 3.1.3 A calculation of percentage frequency for five plant species using five quadrats

Species of plant	Quadrats sampled					Number of quadrats containing the species	Total number of quadrats sampled	Percentage frequency
	A	B	C	D	E			
Trifolium sp.	X	X		X		3	5	(3/5) × 100 = 60%
Lolium sp.		X	X		X	3	5	(3/5) × 100 = 60%
Bellis perennis	X				X	2	5	(2/5) × 100 = 40%
Taraxacum officinale		X		X		2	5	(2/5) × 100 = 40%
Centaurea solstitialis	X		X	X	X	4	5	(4/5) × 100 = 80%
Hyptis capitata		X			X	2	5	(2/5) × 100 = 40%

PA 3.1.2

This method does not take into account how many individuals are present. However, as long as the quadrats chosen are truly representative of the ecosystem being surveyed, this method should give a fair estimate of the plant species present.

Species richness

Species richness is a simple tally of the number of species within a particular area. It does not take into account the number of individuals of each species or how they are distributed in the area. While species richness counts the number of species, it does not tell you what the species are or how valuable they are in maintaining the ecosystem. It may be that 98% of the individuals are of one species and there are only one or two individuals of each of the other species present. This would not be a truly complex ecosystem, as species with very small numbers of individuals can easily become locally extinct.

Calculating species richness

Species richness becomes more accurate as more samples are taken. But how many samples are enough? One way to determine this is to construct a species accumulation curve. This is a graph where the number of quadrats is plotted against the cumulative number of species (Figure 3.1.15).

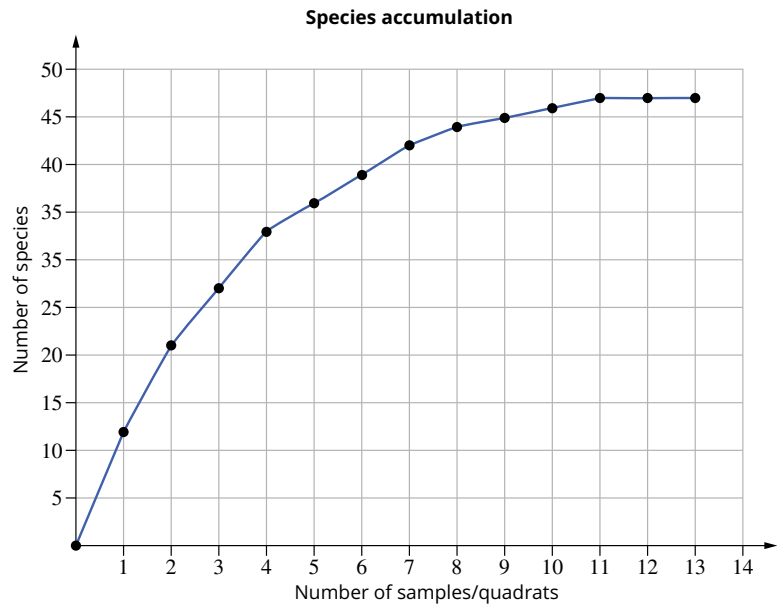


FIGURE 3.1.15 A cumulative species curve levels off when sufficient samples have been taken.

It would be expected that, as each new quadrat is sampled, there will be fewer new species identified so eventually the graph will plateau. At this point sufficient samples have been taken for the researcher to be confident that all but the rarest species have been identified. The greater the species richness of an area, the more samples will be required to ensure an accurate survey.

PA 3.1.1

Species abundance and evenness

Species abundance is the number of individuals of a particular species in the ecosystem under consideration. **Species evenness** or **relative species abundance** is a measure of the number of individuals of that particular species in relation to the total number of individuals of all species in the area.

Evenness is a useful measure of biodiversity as it can show how evenly spread the species **distribution** is. Unlike species richness, species evenness shows whether a few species dominate or whether there are significant numbers of individuals of many different species. For example, Figure 3.1.16 shows two communities of plants that have equal species richness. However, community A has lower species evenness than community B. Although both communities have the same number of species present (species richness), they are more evenly distributed in community B.

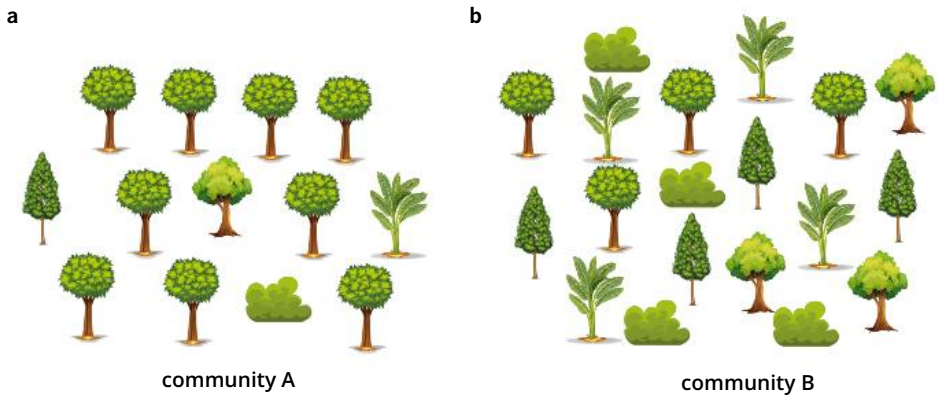


FIGURE 3.1.16 (a) Community A is less diverse than (b) community B because community A is dominated by one species.

Calculating species evenness

Species evenness is a measure of distribution of the species in an area. Both percentage cover and individual population counts can be used to measure evenness. If all sample plots show similar percentage cover of the same group of species, or sampling in different areas gives a similar individual count, then the area is showing species evenness. Distribution of animal species is normally examined using population counts. Animal population sizes are frequently estimated using a method called the Lincoln index (Module 4.3).

Using percentage cover, an average of the cover for each species for the quadrats is calculated and then this can be compared to other areas. A pasture like the one shown in Figure 3.1.7 could be further surveyed using a number of different quadrats. A different pasture would produce different results when surveyed (Table 3.1.4).

TABLE 3.1.4 Species evenness for two different pastures

Pasture 1		Pasture 2	
Species	**Average percentage cover**	**Species**	**Average percentage cover**
Trifolium sp.	2.5%	*Trifolium* sp.	16.7%
Lolium sp.	70.6%	*Lolium sp.*	16.9%
Bellis perennis	4.3%	*Bellis perennis*	16.5%
Taraxacum officinale	15.1%	*Taraxacum officinale*	16.6%
Centaurea solstitialis	1.3%	*Centaurea solstitialis*	17.0%
Hyptis capitata	6.2%	*Hyptis capitata*	16.3%

TABLE 3.1.5 A comparison of species evenness at two sample sites with the same species richness

Area 1		Area 2	
Species	**Species count**	**Species**	**Species count**
A	202	A	32
B	205	B	24
C	196	C	847
D	198	D	15
E	199	E	82

Species evenness can also be estimated using counts of individuals of each species. For example, two different areas were surveyed as shown in Table 3.1.5. Both areas have the same number of individual organisms (1000) and the same number of different species (5) so they have equal richness, but area 2 is dominated by species C, so it has less species evenness than area 1, where all five species show a similar incidence. Two areas can have the same species richness but different species evenness. Area 1 is more even than area 2.

One measure of species diversity is the Shannon–Wiener index. The Shannon–Wiener index can be adapted to provide a measure of species evenness. The formula for species evenness (E) is:

$$E = \frac{-\Sigma\left(\frac{n}{N}\ln\frac{n}{N}\right)}{\ln S}$$

where

n is the number of individuals of one species

N is the total number of individuals across all species

S is the total number of species (species richness)

ln is the natural log.

The calculations for area 2 are shown in Table 3.1.6.

TABLE 3.1.6 Calculation of $-\Sigma\left(\frac{n}{N}\ln\frac{n}{N}\right)$

Species	Species count (n)	$\frac{n}{N}$	$\ln\frac{n}{N}$	$\frac{n}{N}\times\ln\frac{n}{N}$
A	32	0.032	–3.44	–0.11
B	24	0.024	–3.73	–0.089
C	847	0.847	–0.166	–0.141
D	15	0.015	–4.199	–0.063
E	82	0.082	–2.501	–0.205
sum (Σ)	1000			–0.608

The calculation of the Shannon–Wiener index for area 2 is thus:

$S = 5$

$\ln 5 = 1.609$

$$-\Sigma\left(\frac{n}{N}\ln\frac{n}{N}\right) = -0.608$$

$$E = \frac{-\Sigma\left(\frac{n}{N}\ln\frac{n}{N}\right)}{\ln S}$$

$$= \frac{-(-0.608)}{1.609}$$

$$= 0.378$$

If all species in the ecosystem are equally abundant, the value of E (species evenness) will be close to 1. An environment with one very dominant species will provide a value close to 0.

Simpson's diversity index

Both species richness and species evenness only give part of the picture of the biodiversity of an area. A better measure would take both aspects into account. In 1949, Edward H. Simpson determined a method which would take both elements of biodiversity into account. This method is described by the formula now known as **Simpson's diversity index** (SDI). It considers both richness and evenness.

Simpson's diversity index is described by the formula:

$$\text{SDI} = 1 - \left(\frac{\Sigma n(n-1)}{N(N-1)}\right)$$

where

n is the total number of individuals of each species

N is the total number of individuals of all species.

Calculations of Simpson's diversity index produce a number between 0 and 1. A value of 0 indicates a monoculture (only one species is present), whereas a value of 1 would indicate that there is infinite diversity. Values closer to 1 indicate greater diversity than values closer to 0.

Calculating Simpson's diversity index

Consider the results from area 2 in Table 3.1.5. Area 2 contained five species and 1000 individuals spread among those five species. Using a table to calculate $\Sigma n(n-1)$ reduces the chance of calculation errors (Table 3.1.7).

$$N(N-1) = 1000 \times 999 = 999\,000$$

$$\text{SDI} = 1 - \left(\frac{\Sigma n(n-1)}{N(N-1)}\right)$$

$$= 1 - \frac{724\,958}{999\,000}$$

$$= 1 - 0.7257$$

$$= 0.2743$$

The relatively low value for SDI indicates that this area has low diversity. This would be expected, given that there are only five species and the area is dominated by species C.

TABLE 3.1.7 Calculation of $\Sigma n(n-1)$

Species	n	$n - 1$	$n(n - 1)$
A	32	31	992
B	24	23	552
C	847	846	716 562
D	15	14	210
E	82	81	6642
Total (Σ)	$N = 1000$		724 958

WS 3.1.5

SPECIES INTERACTIONS

Species interact within ecosystems. They compete for resources, use each other as food and assist each other. Species interactions can be positive, negative or neutral. In a predator–prey relationship, the relationship is positive for the predator but negative for the prey. In a mutualistic relationship, the interaction is positive for both species. In other relationships, one organism is benefiting but the other is neither benefited nor harmed. You will learn more about the specifics of the types of species interactions in Chapter 4.

Species interactions and their effect are difficult to calculate and many studies are qualitative rather than quantitative. Often the interactions only become clear when changes occur in the ecosystem and the balance is upset. Measuring species interactions involves identifying the relationships and scoring their strength.

This is often done by drawing **networks** that show the interactions. These networks can be unweighted and/or undirected. An **undirected network** does not indicate the type of interaction. An **unweighted network** does not indicate the strength of the interaction between the species (Figure 3.1.17).

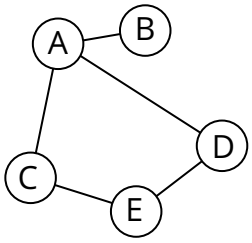


FIGURE 3.1.17 An unweighted and undirected species interaction network.

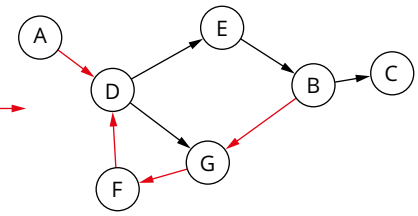
Development of a directed and/or weighted grid requires the development of an interaction matrix, which estimates the size and direction of the impact of the interaction between two species. This can be illustrated in an interaction network. In the example in Figure 3.1.18, species A is having a positive effect on species D, but species D is not influencing species A. Networks can show the strength of the interactions (weight) by the thickness of the arrows between the species.

a

Species	A	B	C	D	E	F	G
A	1	0	0	0	0	0	0
B	0	1	0	0	–0.8	0	0
C	0	–0.1	1	0	0	0	0
D	0.4	0	0	1	0	0.3	0
E	0	0	0	–0.7	1	0	0
F	0	0	0	0	0	1	0.7
G	0	0.8	0	–0.2	0	0	1

Interaction matrix for the community

b



Interaction network for the community

FIGURE 3.1.18 (a) An interaction matrix scoring interactions of species across the top of the matrix with those along the vertical side. (b) An unweighted interaction network developed from the matrix. Positive interactions are shown in red and negative in black.

The large number of species and the complexity of interactions in most ecosystems means that identifying and scoring the strength of species interactions requires large amounts of data to be collected over long periods of time. Species interactions are not always consistent through time. For example, in southern Australia, rodents are frequently predated by snakes in the summer, but in the winter the snakes hibernate so few, if any, interactions between snakes and rodents occur.

Multiple methods can be used to measure species interactions. For example, ecologists used several methods to measure species interactions in the two case studies below. Depending on the type of ecosystem and organisms present, as well as time and financial resources, scientists may select different methods.

CASE STUDY 3.1.1

Species interactions in Shark Bay

Shark Bay, Western Australia, is the site of the famous Monkey Mia beach. Public interest in this area has stimulated considerable study of the ecology of this area. Studies have been undertaken by the Shark Bay Ecological Research Project of the populations of dolphins, sea turtles, sharks and dugongs, along with the availability of the fish, invertebrates and sea grasses that provide the major food sources of the large vertebrates. Dolphins, sea turtles and dugongs in Shark Bay are also preyed upon by the larger shark species, especially tiger sharks.

At least 24 species of shark inhabit Shark Bay, but by far the most abundant species is the tiger shark (*Galeocerdo cuvier*). Tiger sharks have a significant effect on the abundance and distribution of species in Shark Bay.

Tiger shark numbers in the bay are strongly influenced by seasonal effects. Tiger shark numbers are significantly lower in colder months than in warmer months. For research purposes, tiger sharks are caught and tissue samples are taken before they are released. Far fewer sharks are caught during cold periods of the year (Figure 3.1.19).

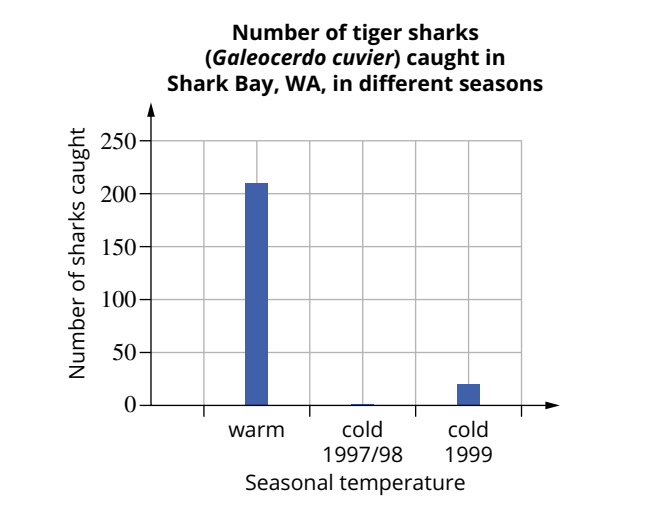


FIGURE 3.1.19 The variation in tiger sharks caught seasonally in Shark Bay, Western Australia.

The activities of a number of species are influenced by the sharks. Dugongs and green turtles both feed on the seagrasses of Shark Bay. The turtles also feed on comb jellies and other invertebrates but the dugongs' diet is almost exclusively seagrass.

Snipping the leaves of the seagrasses causes very little harm to the seagrass population, but when the dugongs dig out the roots for food, the seagrass population is reduced in size. The impact of the dugongs on both the richness and abundance of the seagrass species can be significant due to their large appetites. Shark Bay contains 12 species of seagrass, belonging to at least three different genera. During the warm summer months, the dugongs feed on seagrasses in the shallow, eastern areas of the bay.

Studies have shown that in some areas the dugongs remove up to 50% of the seagrasses, resulting in a significant reduction in abundance of these species. As the seagrasses also shelter other species, providing camouflage and protecting them from predation, this loss is likely to impact on other species present in the bay.

The dugongs prefer the rhizomes that form the root system of the seagrass, but will crop the leaves when sharks are around. Rhizomes are a better-quality food source, but digging for them puts the dugongs at greater risk from shark predation as it makes observing sharks more difficult. When sharks are common, the dugongs feed on leaves because it is safer. The studies in Shark Bay also showed that the dugongs tend to feed along the edges of the seagrass fields, close to deep water, in the warmer months. If the dugongs swam in deep water, they were likely to escape from an attacking shark.

As the water temperature drops in the winter, the dugongs move to the outer areas of the bay. In these areas the dominant species of seagrass is *Amphibolis antarctica*. This is a less nutritious food source, but the water is warmer than in the shallows.

The dugongs are a major food source for tiger sharks and the sharks are most likely to catch dugongs when they are feeding in the shallow seagrass beds during the summer. It has been hypothesised that the dugongs attract the tiger sharks to Shark Bay.

Review

1. Explain the dugongs' effect on species abundance and distribution in Shark Bay.
2. Identify one biotic and one abiotic difference between the ecosystem of Shark Bay in winter and summer.
3. **a** Identify two species within Shark Bay that are in competition.
   b State one resource for which they compete.
4. Name the relationship that exists between comb jellies and turtles.

CASE STUDY 3.1.2

Species interactions on Christmas Island

Christmas Island is an Australian territory 1550 km off the north-west coast of Western Australia. It has a rich biodiversity and contains a number of unique species. One species endemic to Christmas Island is the red land crab. The only other place red land crabs are found is the nearby Cocos Islands.

Adult red land crabs live in the tropical rainforests of Christmas Island. Each year, at the beginning of the wet season, they migrate to the coast to breed (Figure 3.1.20). The eggs hatch in the ocean, where the larvae remain for about a month. The young crabs then also move into the rainforests of Christmas Island.

FIGURE 3.1.20 The annual migration of red land crabs on Christmas Island, when millions of crabs make their way to the coast.

Red land crabs play an important role in the ecosystem. They scavenge for food on the forest floor where they eat leaf litter, seeds and dead animals, facilitating nutrient turnover and enriching the soil. The crabs also prey on small invertebrates, most notably the giant African land snail, which is an introduced pest. The crabs dig out burrows to live in. This turns over, aerates and stops compaction of the soil. Aerated soils increase oxygen supplies to plant roots and assist their growth. The population size of the crabs has been estimated to be around 43 million. They make a significant contribution to soil condition and also to maintaining the biology of the rainforest.

Unfortunately, yellow crazy ants were accidentally introduced onto Christmas Island sometime between 1915 and 1934. This invasive species kills the crabs by spraying them in the eyes and joints with large amounts of formic acid. The crabs are immobilised and eventually die. It has been estimated that the population of crabs has decreased by up to 30% since the introduction of the ants, with most of the most serious effects occurring since the 1990s.

The ants had little impact until they began forming supercolonies that cover large areas and consist of more than 7.5 billion individuals. The first supercolony was discovered in 1989 and since then many more have formed. These supercolonies have had a major impact on species abundance and distribution on Christmas Island. Scientists are not sure why the ants only began forming supercolonies in 1989.

The ants have a symbiotic relationship with a species of yellow lac scale insect. Symbiotic relationships are close, long-term relationships between two species. In the case of the ants and scale insects, the insects suck sap from the trunks of trees and produce a waste product called honeydew that is rich in sugar, which the ants use as food. The ants farm the scale insects for this food supply. This relationship has allowed both the ants and the scale insects to increase in abundance to the detriment of other species.

The ants are also predators. They feed directly on any small invertebrates that they find. The areas inhabited by supercolonies are almost entirely devoid of insects, spiders, millipedes and other small invertebrates. The ants also scavenge the bodies of larger organisms, such as the red crabs. As most susceptible animal life is quickly consumed by the supercolony, without the farming of the scale insects these colonies would collapse. The voracious appetite of an ant supercolony greatly reduces the biodiversity of the areas they inhabit.

Areas invaded by ant supercolonies show distinct differences to untouched areas. Where ant supercolonies exist, more weeds and dense seedling thickets grow where, previously, the crabs ate seeds, seedlings and weeds. The soil is more compacted due to the loss of crab burrows. Leaf litter is much thicker and nutrient cycling is reduced. Also, the photosynthetic rate in some trees is reduced as a result of mould growing on their leaves, which blocks light penetration. The growth of the mould is facilitated by excess honeydew from scale insects dripping onto the plants' leaves and providing food for the mould.

These factors result in a reduction in biodiversity, as a few invasive weeds take over from the diverse natural vegetation profile and pest plants, such as the stinging tree, become more abundant.

The rainforest has become a patchwork, depending on whether the ants have invaded an area or not. In ant-free areas, there is high species richness and native species are relatively abundant. In ant-infested areas, the species richness is lower and the native species that remain have much lower abundance.

Controlling the ants

The impact of the yellow crazy ant has been so significant that research is underway to find a method of controlling their numbers. While eradication is unlikely, it may be possible to stop the yellow crazy ants forming their destructive supercolonies.

An experiment was undertaken to evaluate the importance of the yellow lac scale insect to the maintenance of the ant supercolonies. The scale insects infest branches high in the canopy of large trees and the ants live on the ground. Ants move up and down the trees, continuously bringing honeydew and any prey that they find back to the nest.

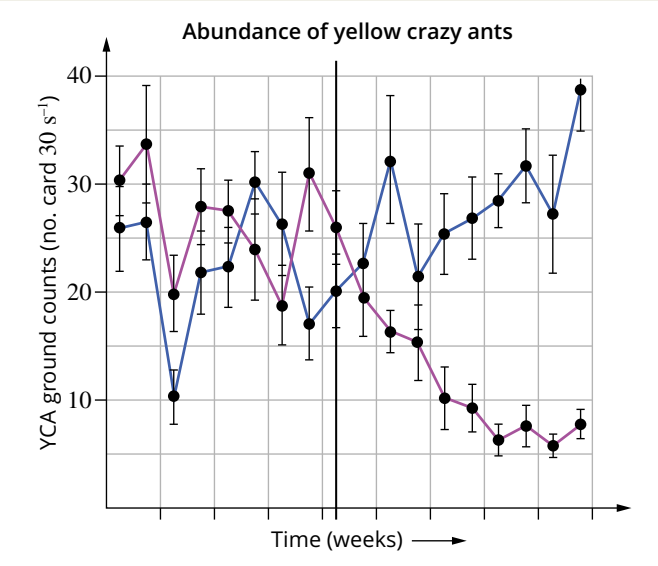


FIGURE 3.1.21 The abundance of yellow crazy ants decreased significantly when access to the yellow lac scale insect was denied. The blue line shows ant activity in the control colony. The purple line is the experimental colony.

Scientists looked at two similar supercolonies: the control colony was left untouched, but in the other all of the trees were banded so that ants could no longer move up or down the trunk. Ants returning from the treetops were examined and then gently brushed off to join the rest of the colony. Only a small number of ants returning from the canopy carried prey. The vast majority were swollen with honeydew. Examining and brushing off the ants continued until there were no more returning ants, and thus no ants remained in the canopy.

The control colony remained unchanged in size throughout the experiment, but the experimental colony experienced collapse and decreased in size by 80% in weeks, demonstrating how the relationship with the scale insects supported the abundance of the ants. The change in the ants' activity due to reducing abundance is shown in Figure 3.1.21.

This data suggested a method of controlling the ant colonies by reducing the scale insect population. In early 2017, after considerable research, a micro-wasp, which lays its eggs exclusively in yellow lac scale insects, was released onto Christmas Island.

Review

1. Propose why the scientists needed to examine the ants returning from the canopy before brushing them off.
2. Explain why the scientists brushed the ants off to return to the nest rather than killing them.
3. Explain the likely impact of the micro-wasp on Christmas Island biodiversity.
4. **a** Describe the relationship between the red land crabs and the rainforest plants in undisturbed areas.
   b Name this relationship.
5. Identify a pair of organisms on Christmas Island, other than red land crabs and rainforest plants, which have a relationship which is:
   a positive for both organisms
   b positive for one species and negative for the other
   c positive for one species and neutral for the other.

Measuring species interactions in Shark Bay and on Christmas Island

In the case of Shark Bay, several methods were used to identify and quantify the interactions between the species present. When turtles were captured during population counts, the number of shark-bite injuries on the turtles were tallied as a measure of the number of interactions between sharks and turtles. Tissue samples were also taken and the concentrations of stable isotopes of nitrogen were analysed. Proportions of stable isotopes of oxygen, carbon, nitrogen and sulfur in tissue samples can all be used to provide information about predation within an ecosystem.

For example, there are two stable isotopes of carbon: carbon-13 and carbon-14. The ratio between these two isotopes in the tissues of an organism is a guide to the food source of the organism. Large differences in the ratio between two species show that they are eating different things. It can be used to determine which organisms are herbivores and which are carnivores. It can also assist with determining where in a food chain a carnivore can be found.

Another isotope used to determine feeding relationships is nitrogen-15. In Shark Bay, analysis of nitrogen from the tissues of turtles was used to determine which turtles feed primarily on comb jellies and which feed primarily on seagrass. Nitrogen-15 concentration was higher in comb jelly feeders (carnivores) than in seagrass feeders (herbivores). This is a typical pattern. Nitrogen-15 concentration is found in greater concentration higher up a food chain.

Another method used by scientists studying Shark Bay to determine species interactions was to attach cameras to organisms such as turtles and sharks to determine visually how species were interacting. The number of minutes of interaction in comparison to the total number of minutes of the video gives a measure of the amount of interaction.

On other occasions, exclusion experiments such as the experiment undertaken to understand the interaction between yellow crazy ants and yellow lac scale insects on Christmas Island are undertaken. Such experiments can also be undertaken, with appropriate permission, in rock pools. A species can be removed from a rock pool and the subsequent effects identified and measured.

Many ecosystems and many individual species in Australia are protected so any study that could change the behaviour of native flora or fauna or may somehow result in harm to an organism should not be undertaken without checking with the appropriate authorities.

3.1 Review

SUMMARY

- Biodiversity can be characterised in three ways: genetic, species, and ecosystem or biome.
- Each biome has its own unique set of physical and climatic characteristics, and these determine the species that live there.
- Biodiversity can be measured using various measures, including percentage cover, percentage frequency, species richness, species abundance and evenness.
- Species evenness can be calculated using the Shannon–Wiener index.
- Species diversity can be calculated using Simpson's diversity index.
- Species interactions are a major determinant on the species present in an area. They may be positive, negative or neutral.
- Species interactions can be scored on an interaction matrix and displayed as an interaction network.
- Relationships between species are sometimes difficult to establish and many methods are used, including direct observation in person, by air or boat, or by attaching monitoring equipment such as cameras to individual members of a species. Biochemical studies of tissue samples or waste products can also be used.

KEY QUESTIONS

Retrieval

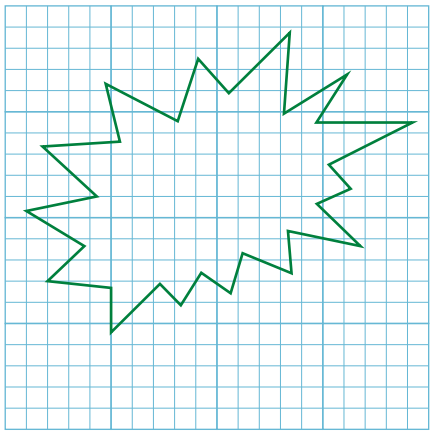
1 Define biodiversity.

2 Describe the difference between species richness and relative species abundance.

3 Describe how percentage coverage can exceed 100%.

Comprehension

4 Calculate the percentage cover of the plant shown in this 1 m × 1 m quadrat. Only count small squares if they are covered more than 50%.



5 Explain how poor quadrat selection can result in inaccurate understanding of biodiversity.

Analysis

6 During a 2003 study in the Caribbean, the mass of fish in ecosystems near both mangrove-rich and mangrove-sparse coastal regions was determined. The results of that study are shown in the table below. The scientists performing the study were using fish mass as a measure of abundance, with higher mass indicating a higher abundance.

Mass of fish in ecosystems near mangrove-rich and mangrove-sparse coastal regions

Species surveyed	Mangrove-sparse area fish mass (kg km^{-2})	Mangrove-rich area fish mass (kg km^{-2})	Mean mass of individual fish (kg)
striped parrotfish	1530	2170	6.0
bluestriped grunt	288	447	0.65
schoolmaster snapper	1767	1898	0.6
French grunt	1308	1643	0.7
white grunt	523	863	0.65
yellowtail	3098	6715	3.8

a Calculate the mean number of individuals of each species per km^2 for each species in each area.

b Calculate the species evenness of the two areas.

c Comment on species richness and evenness in the two areas.

continued over page

3.1 Review *continued*

7 A survey of an area produced the species counts shown in the table below.

Species	Number
A	45
B	13
C	82
D	60

Comment on the biodiversity of this area using Simpson's diversity index.

8 Calculate species diversity, using Simpson's diversity index, for the data in the table below, which shows individual number counts for each species in a given area.

Species	Number
A	37
B	29
C	43
D	34
E	27

9 **a** Construct a species interaction network from the following matrix.

Species	A	B	C	D	E
A	1	0	0	0.3	0.5
B	0.5	1	–0.8	0	0
C	–0.2	0	1	0	–0.7
D	0.5	0.8	–0.3	1	0
E	0	0	0	–0.4	1

b Describe the relationship between species A and species D.

3.2 Biodiversity and the abiotic environment

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- identify relevant abiotic factors affecting diversity in terrestrial environments
- measure factors such as salinity, turbidity and pH using appropriate measuring tools
- identify and measure factors affecting aquatic environments
- compare ecosystems
- explain how and why ecosystems vary across both spatial and time scales.

The main focus of this module is the identification and measurement of the abiotic factors that influence biodiversity in both terrestrial and aquatic environments. In this module, you will also consider how ecosystems vary across both spatial and time scales.

TERRESTRIAL ENVIRONMENTS

The abiotic characteristics of an ecosystem determine the species that live there. For example, the cold temperatures which prevail for most of the year in alpine regions means that reptiles are very rare in those areas. Reptiles are ectothermic and are unable to maintain their metabolism at high altitudes.

Among the most influential of the abiotic factors are those that make up climate. Climate is one of the major defining characteristics of biomes.

Climate

Climate encompasses a number of factors including precipitation, temperature, wind direction, cloud cover and sunshine. All of these will affect the type and **abundance** of species present.

Wind direction can be a major determinant of biodiversity not only because it can determine factors such as salinity near the coast, but also because it has a major influence on precipitation in many regions. Along the coast, winds blow salt spray into the surroundings. Salty environments are very challenging for most plant species and so the diversity close to the shoreline of terrestrial organisms may be low. A little further inland, however, the winds are beneficial. As the air masses pass over the ocean, large amounts of water are evaporated. This is fresh water, which forms clouds. As the winds pass over the land, the water in the clouds condenses, resulting in rainfall. In the tropics, where temperatures are higher, the amount of water evaporated into the clouds is much greater, so rainfall is greater than in areas of lower temperature. As all living things require water for life, a larger supply of water tends to increase biodiversity.

Wind also contributes to the water cycle and is highly influential in plant transpiration. Transpiration is the process of water movement through plants from the soil, into the plants via the root system, to the extremities such as leaves, flowers and stems where it evaporates from the plant. The details of this process are explained thoroughly in Module 5.4 of *Pearson Biology 11 Queensland*. Depending on the type of plant, up to 99% of the water passively absorbed into plants is lost through evaporation. Wind is highly influential in maintaining or increasing the rate of transpiration in plants by causing evaporation from the leaf surface. Plants require water for turgor, photosynthesis and respiration. Plants maintain an intricate balance between transpiration rate, turgor and metabolism that incorporates climatic variables, such as wind.

In plant cells, turgor is a state of high internal fluid pressure that is the result of the osmotic intake of water.

The interaction of wind with plants, the primary producers of an ecosystem, support various other interactions, for example, energy flow, the water cycle, nutrient resources, living and nesting habitats, camouflage and protection and more. Ultimately, climate influences biodiversity.

Climate also has an influence on soil. Tropical climates are warm and wet. Plants tend to receive a lot of sunlight. Under these conditions, plants grow very well and organic matter decays quickly. However, tropical soils are generally relatively nutrient-poor as the high rainfall causes substantial **leaching** of the soils. Leaching occurs where rainfall dissolves and washes away water-soluble nutrients from the upper layers of soil.

Despite the poor soils, plant growth in tropical regions is still luxuriant. The abundance of water and sunlight, along with the warmer temperatures, all support photosynthesis and plant growth. Dense plant growth increases biodiversity, not only because there are more types of plants, but also because plants support food chains. Many plants allow for the existence of many animals.

Both desert and arctic climates receive little rainfall. Plant growth is slow and enrichment of the soil with organic matter is slow or mostly absent. The mineral content of the soils tends to be higher than in the tropics as the lack of rain reduces leaching.

Temperate climates tend to be the best for soil formation. There is sufficient rain for both chemical weathering of bedrock and plant growth through the provision of minerals. There is also sufficient rain for decay to occur but not so much rainfall that leaching is a problem. This results in the formation of deep, nutrient-rich soils that support plant growth.

While warmer climates tend to support biodiversity, colder climates tend to reduce it. All ecosystems depend on their producers to support the other organisms. Cold temperatures slow the metabolism of most living things, including plants. Plants living in cold environments tend to grow either very slowly or very sporadically. The harshness of these cold environments allows only a few species of plants to survive and flourish. As a result, food supplies for animals and other heterotrophic organisms are scarce. Limited food resources result in limited biodiversity.

Heterotrophic organisms obtain nutrients from other organisms.

Data about a study site, such as temperature and rainfall, will vary through the seasons and this data must be collected and considered. Rain gauges (Figure 3.2.1) and temperature probes can be used. Climate data is also collected by the weather bureau in each Australian state and is available to the public for most areas. Depending on the proximity of weather stations to the study site, data from the Bureau of Meteorology may not accurately reflect conditions at the study site.

FIGURE 3.2.1 A simple rain gauge can be constructed using a retort stand, clamps and a graduated test tube or a measuring cylinder. Data should be collected daily.

Bureau of Meteorology data is still helpful, because it provides long-term data. Changes in trends over time, which may not be obvious in the short term, can be observed. Ideally, both short-term localised data and longer-term trend data should be obtained and considered.

Availability of a regular supply of water can affect both species richness and abundance. Table 3.2.1 shows the difference between the arid desert of Kati Thanda–Lake Eyre and the Daintree rainforest.

TABLE 3.2.1 Species richness comparison between Kati Thanda–Lake Eyre during a dry period and the Daintree rainforest

Animal groups represented	Kati Thanda–Lake Eyre and surrounds (number of species)	Daintree rainforest and surrounds (number of species)
birds	66	430
amphibians (frogs)	4	54
reptiles	21	193 approx.
insects	167 approx.	12 000+
mammals	21	116 approx.

A significant difference between the Daintree and Kati Thanda–Lake Eyre is the amount of water each area receives. The Daintree experiences large regular falls of rain. The inland of Australia receives only intermittent rainfall, experiencing long periods of drought followed by occasional periods of flood.

Soils

Soils are formed when the underlying bedrock is weathered and broken down into grain-sized particles, so the underlying rock has a large influence on which plants can grow in an area. The types of plants present will determine which animals can also live in an area, so soils are a determinant of the type of ecosystem in any particular region.

The major determinants of soil quality in Australia are:

- thickness
- structure and porosity
- pH
- nutrient content
- salinity.

In much of Australia, the soils are very ancient, shallow and nutrient-poor. Acidification and rising salinity are also an issue. Many soils are especially deficient in nitrogen. The most productive soils consist of grains of varying size along with a mixture of minerals and decayed organic materials.

Soil thickness increases over time as weathering of bedrock occurs and materials accumulate over the rock layers. Soils show a layering, with each layer, called a **horizon**, having particular characteristics of nutrient content, particle size and structure (Figure 3.2.2). The type of bedrock weathered and related subsoil is a major determinant of the mineral nutrients present and the structure of the top soil. This in turn influences the types and numbers of plants that can grow in any area. For example, soils derived from basaltic bedrock are generally rich in calcium, iron and magnesium.

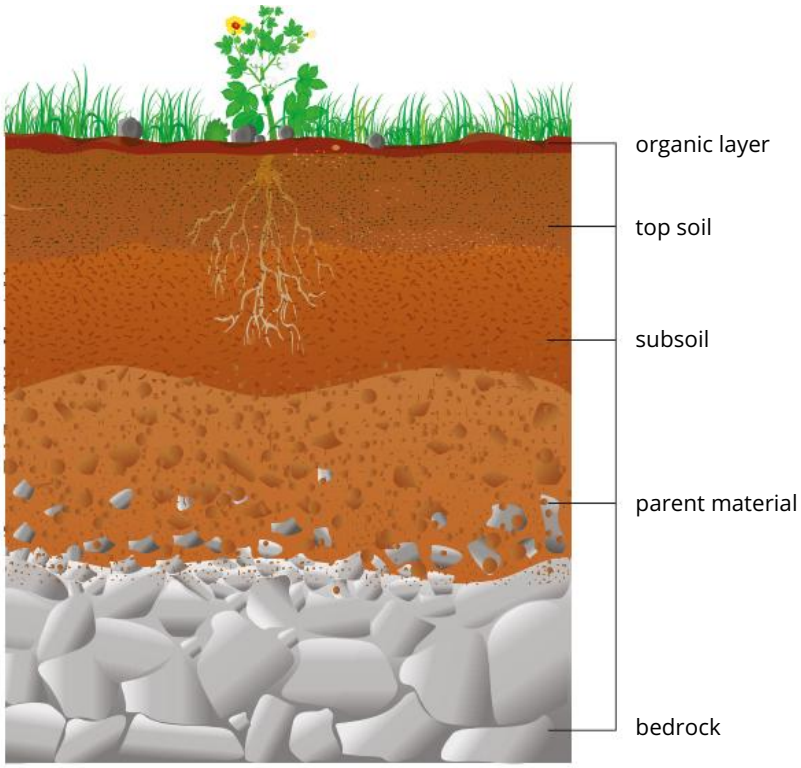


FIGURE 3.2.2 A soil profile showing the typical horizons found as soils develop from bedrock.

FIGURE 3.2.3 Steep slopes are less vegetated than flatter areas.

Slope, climate and vegetation can enhance or inhibit the process of soil formation. Areas prone to erosion, such as those with a steep slope (Figure 3.2.3), tend to have thinner soils because water run-off washes away the soils, sometimes as fast as they build up. This is a challenging environment for plants. There is little anchorage for their roots and the thin soils tend to lack nutrients. Steep slopes also tend to be water-deficient environments due to the rapid water run-off, and this deprives plants of the water they need for photosynthesis and transpiration.

If rainfall is high, the upper layers of the soil are subject to leaching. Leached soils are common in tropical rainforests.

Soils that are only leached are not as challenging as those on steep slopes but they still lack mineral nutrients, especially the water-soluble nitrates and phosphates that many plants need to survive and thrive. Plants need a good supply of nitrogen and phosphorus to build proteins and nucleic acids.

Other factors in the rainforest help to offset the harmful effects of soil leaching on plant growth. One important factor in maintaining soils in rainforests is the high levels of decomposing organic material, called **humus**, in the upper layers of the soil. Decomposition is fast due to the warm moist environment and this helps to increase the availability of nutrients that enhance plant growth.

Soil structure consists of the material that makes up the solid part of the soil, along with the spaces between the solid materials. The spaces are called pores. To a large extent, the number and arrangement of the pores determines the productivity of the soil. It is in the pores that air and water is stored, organisms like worms live, and organic matter accumulates. The size of the pores also determines how easily water penetrates the soil and how easily it drains (Figure 3.2.4).

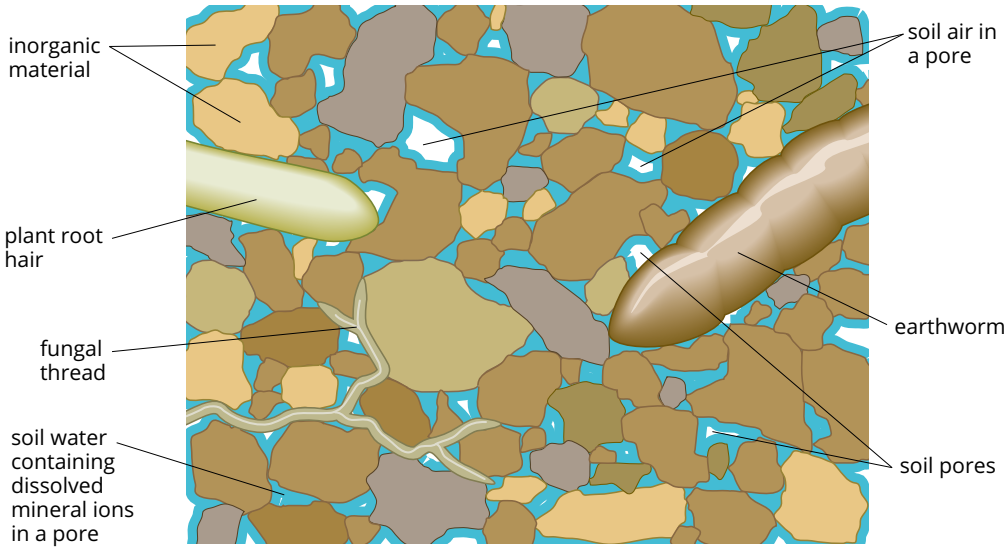


FIGURE 3.2.4 The structure of soil includes pores, inorganic products of the weathering of bedrock, and organic materials.

The more densely packed the materials are in the soil, the fewer pores there are, which results in fewer air spaces and therefore less oxygen. Penetration by roots becomes more difficult, as there are fewer organisms living in the soil and water and air do not penetrate as easily. Soils that are predominantly clay do not absorb water easily and also do not release it once absorbed. The grains of clay are relatively small and packed closely together, so they also lack pores. Clays are therefore low in oxygen and may also lack nutrients. Sandy soils also tend to be nutrient-depleted but they are extremely porous and allow water in and out quickly (Figure 3.2.5).

The best soils are loams. These have a range of particle sizes and absorb, retain and release water to plants readily. Loams also tend to be high in mineral nutrients.

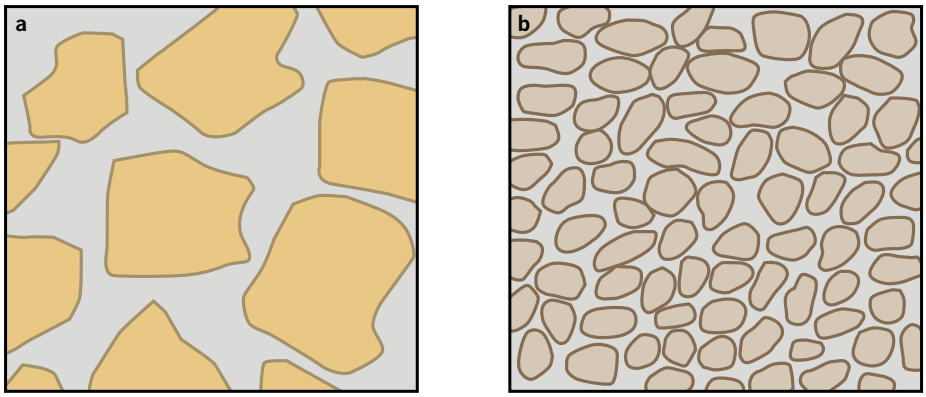


FIGURE 3.2.5 (a) Sandy soils have larger particles and larger pores than (b) clay soils. Large pores can hold the water and air needed by plant roots for growth.

Soils also range in pH. Some soils are acidic, some are alkaline, and some are neutral. Different plants grow in different soils. Some plants are able to tolerate a large range of soil pH levels, while others are limited in the pH in which they can grow. *Hydrangea* can grow in soils with a range of pHs, but the pH affects the colour of its flowers (Figure 3.2.6).

Plant growth is generally optimal at pH levels between 5.5 and 8. At pH levels of less than 5.5, soil nutrients such as nitrogen, phosphorus and calcium, which are necessary for plant growth, become less available. Also, and possibly most significantly, aluminium in the soil becomes soluble. Aluminium causes a large reduction in root growth, which slows or stops overall plant growth. This affects most plants, so the plant diversity of an area will be lower if the soils are acidic. At pH values greater than 8, soil nutrients such as iron, manganese, zinc and phosphorus become less available to the plants. This retards growth. Figure 3.2.7 summarises the availability of different essential plant nutrients as soil pH changes.

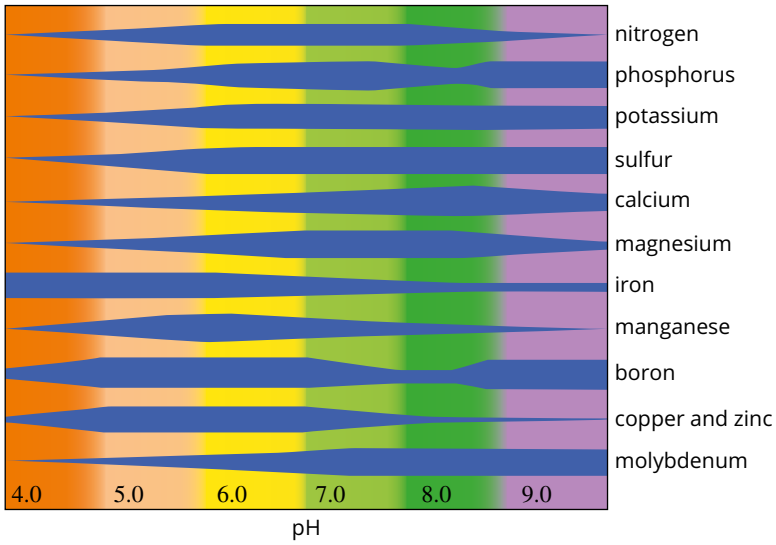


FIGURE 3.2.7 The effect of pH on the availability of plant nutrients. The width of each bar indicates the relative availability of each nutrient.

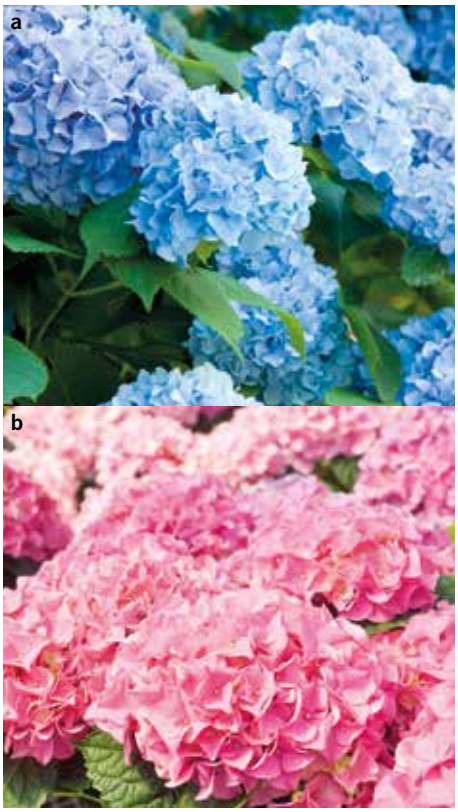


FIGURE 3.2.6 *Hydrangea* has (a) blue flowers in alkaline soils and (b) pink flowers in acidic soils.

FIGURE 3.2.8 A sundew digesting an insect in order to obtain enough nitrogen to make its required proteins and nucleic acids.

Many Australian soils are nitrogen-deficient. Most plants need plentiful supplies of nitrogen, primarily as nitrates, and are at a disadvantage in these areas. Only a limited range of plants can cope with nitrogen-poor soils. Different plants use different strategies to cope with nitrogen-poor environments. One strategy involves forming symbiotic relationships with bacteria that absorb nitrogen from the air and make it available to the plant (Module 4.1). Sundews are insectivorous plants and obtain extra nitrogen from digesting insects and spiders (Figure 3.2.8). Sundews are widespread in Australia but often go unnoticed because of their small size. Their presence is a strong indicator of a nitrogen-poor soil, as they are not good competitors but rely on being able to live in areas that do not meet the needs of other plants.

Finally, large areas of Australia have naturally saline soils that create an environment that tends to be dehydrating for plants. The saline soil can be buried over time as layering from weathering continues, allowing a greater range of plants to grow. Unfortunately, in many areas where the salty layers were buried in the past, irrigation of land by humans has caused salt from these deeper layers to dissolve and rise to the surface, making the surface layers of soil saline and unsuitable for many plant species.

Plants are the producers for most terrestrial ecosystems. Soil determines which plants grow and how well-vegetated the area is. As all animals in terrestrial ecosystems rely on plants for numerous resources, soils are a major factor in the biodiversity of a region.

Measuring soil characteristics

Soil type has a significant impact on the plants present in any particular area. The soil's structure, depth, nutrient content and pH should be assessed.

An auger should be used to prepare a soil core for the area being researched. This will allow examination of the subsoils (Figure 3.2.9). Subsoils should be considered as plant roots often penetrate into these levels and therefore are affected by their condition.

FIGURE 3.2.9 A soil core showing that soil composition changes between the surface and lower layers.

Determining soil structure

The mix of grain sizes determines the porosity, drainage and nutrient-holding capacity of the soil. As all of these impact the variety of plants present, all should be assessed and noted.

The grain mix of soils can be determined by drying out a sample and passing the dry mix through a series of sieves to determine the percentage of each sized particle. The soil can then be classified using a chart such as the one shown in Figure 3.2.10.

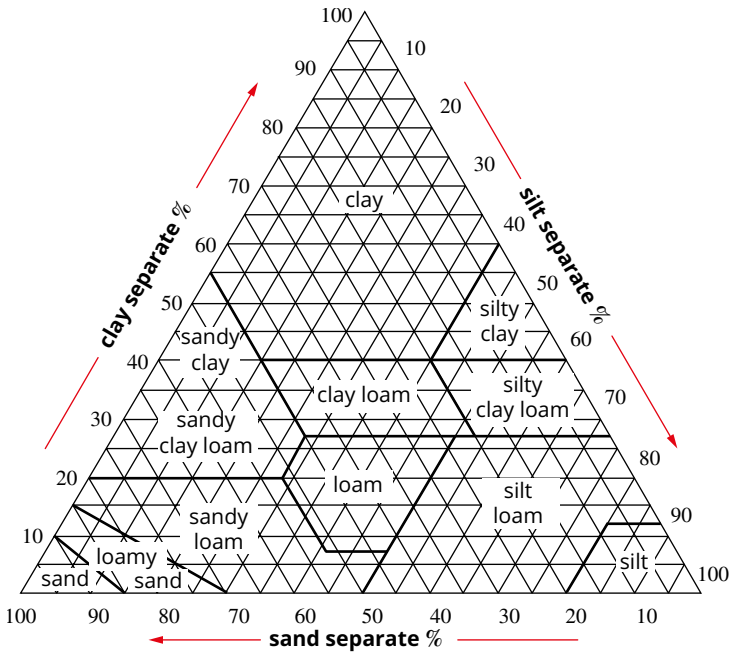


FIGURE 3.2.10 The classification of soils based on the mixture of different sized soil particles.

Soil classification is based on the mixture of sand, silt and clay. Any two of the grains can be used to determine soil type. To interpret the diagram, choose a relevant component percentage, such as 40% sand, and follow the grid line until it reaches the percentage of the second grain size. For example, if a soil is 20% sand, 35% silt and 45% clay, start on the sand axis at 20% and follow the grid line until it reaches the 35% grid line for silt. The soil is classified as a clay.

For another example, imagine a soil sample made up of 30% sand, 30% clay and 40% silt. Find 30% along the bottom (sand) line, and follow the slanted line up and to the left. Stop at the horizontal line for 30% clay, and find the soil type: clay loam.

Measuring soil salinity

Soil salinity is measured by determining its electrical conductivity. The units used are deciSiemens per metre ($dS\,m^{-1}$) or microSiemens per centimetre ($\mu S\,cm^{-1}$). A simple field test can be undertaken, although it may have a margin of error (uncertainty) as high as 10%.

As water movement in the soil affects salinity, the first step is to identify the type of soil: loam, clay, sandy clay etc. A conversion factor must be applied to the test results to account for the differences between soil types. The conversion factors are given in Table 3.2.2. They have been calibrated for soils from the east coast of Australia.

TABLE 3.2.2 Conversion factors for soil salinity tests

Soil type	Multiplication factor
sand	18
sandy loam	13.8
loam	9.5
clay loam and light clay	8.6
medium or heavy clay	7

To complete the field test, a calibrated salinity meter is required. Collect a sample of the soil to be tested and allow it to dry out. Then crush it sufficiently to break down any large lumps. Remove any stone or organic material. Take 20 g of soil and add 100 mL of distilled water into a vessel with a lid. Seal the vessel and shake the mixture for at least three minutes. High clay mixtures may require substantially more shaking for accurate results. Let the mixture settle for one minute before opening the container, inserting the probe and taking a reading. The probe should be placed only in the liquid part, not the solids that have settled to the bottom of the container. Electrical conductivity is different in water and soil, so the reading on the meter should be multiplied by the appropriate adjustment factor from Table 3.2.2.

Determining soil colour

Along with structure, colour is useful in assessing soils. Colour is an indication of nutrients present. There are three main colours: black, white and red.

Soils vary across Queensland due to the varying conditions of bedrock, climate and life. Figure 3.2.11 shows the range of soils found in Queensland.

FIGURE 3.2.11 The variety of soils seen across Queensland. Different colours reflect different conditions of their formation.

Black soils have a high proportion of organic material. While this is good for plant growth, if there is too much humus, the soils tend to become waterlogged. In waterlogged soils, the pores fill with water and reduce the oxygen-carrying capacity of the soil.

White soils consist mostly of silicates. They tend to be low in nutrients, especially iron and manganese. Many white soils are the result of leaching. Leaching occurs when rainfall is high and water-soluble minerals are washed out of the soil. Some white soils are also high in salt.

Red soils generally have good drainage, which means water availability to plants can be low. They are high in iron and provide phosphorus to the plants. Some are also high in aluminium oxides. The iron in these soils, along with the high oxygen content, causes the formation of iron oxide that gives the soil its red colour.

Other colours are a mixture of the three colours and have a blend of properties. For example, greenish soils are high in iron and manganese but, due to poor drainage, tend to be waterlogged. Waterlogging reduces available oxygen and causes the iron and manganese to form green-coloured minerals rather than the red ones typical of better drained soils.

Determining soil pH

Soil pH should also be assessed and recorded. Litmus paper or indicator solutions can be used, but the most accurate method of determining soil pH is with a pH probe connected to a computer.

Area

Over the last few thousand years, humans have changed the landscapes of most of the continents, including Australia. Where once there were huge forests that covered thousands of square kilometres there are now, in many cases, only small areas of forest left. This is also true of many other habitat types.

Small areas often have reduced biodiversity because the competition for resources is more intense than in larger areas. This means that species which live in a large area and may be low in numbers would most likely disappear in a smaller area.

Even when the species richness is the same in a small area as a large area, there is often a loss of diversity because genetic diversity is usually lower in the small area due to the smaller population sizes. Genetic diversity will be explored in detail in Chapter 9.

Large-scale ecological studies of the effects of area size on biodiversity are rare due to the practical problems such studies present. However, one such study has been undertaken in Manaus, Brazil. A part of the Amazon rainforest was released by the government to be cleared for farming, but areas were to be set aside as refuges for native wildlife. Scientists convinced the parties involved to make these refuges rectangles of specific areas. The plots ranged in size from 1 hectare to 100 hectares.

When the forest surrounding the refuges was cleared and crops were planted, a long-term investigation called Biological Dynamics of Forest Fragments Project (BDFFP) began. The remaining forest patches have been surveyed continuously since 1979 when the project began. During the survey, it was found that the smallest areas had the least diversity. Many effects have been observed. For example, the 1-hectare areas could not support larger herbivores and the predators that fed upon them, so these species either emigrated or starved. With the loss of the larger predators, smaller herbivores increased in number, including the small species that fed predominantly on seeds. The increased number of animals feeding on seeds led to a reduction in the germination rates of the seed-bearing plants and a consequent loss of some plant species. Other surveys in the study area discovered that the forest fragments of less than 100 hectares lost half of their bird species within 15 years.

Other smaller-scale studies have added support to the findings of the BDFFP. One 2013 study looked at the number of bird species found in parks in Spain compared to the size of the park. The results of the study are illustrated in Figure 3.2.12.

One hectare is 10 000 m^2. There are 100 hectares in 1 km^2.

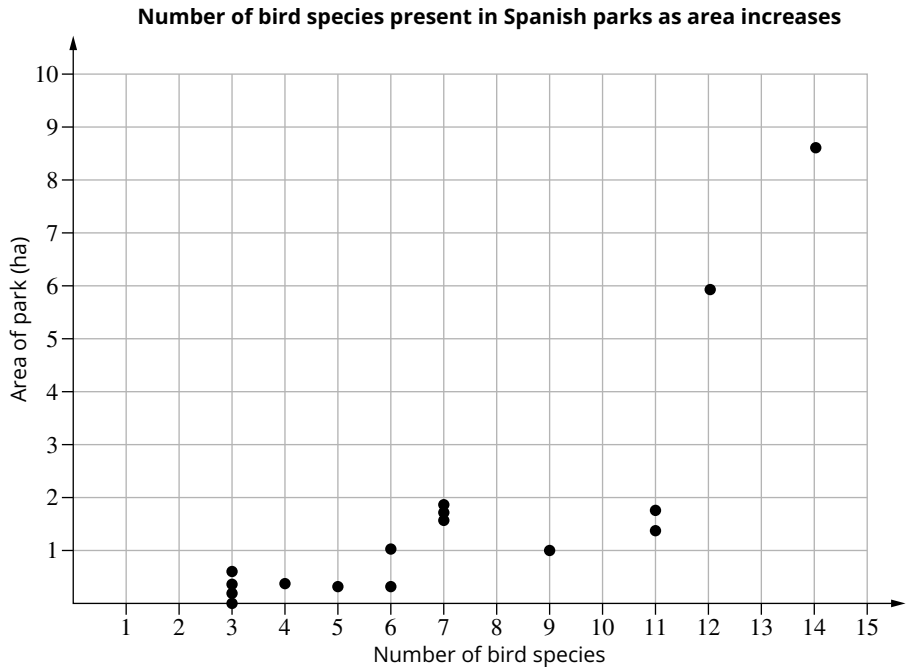


FIGURE 3.2.12 The results of a study in Spain researching the influence of park size on bird species richness.

AQUATIC ENVIRONMENTS

Aquatic environments are as variable as terrestrial environments. Each has its own unique set of abiotic characteristics, which then determine the abundance, evenness and richness of the species present. There are two main types of aquatic environment: freshwater and marine. Freshwater environments range from shallow intermittent lakes to small fast-flowing creeks and huge slow-moving rivers. Temperature and other elements of climate are largely dependent on the surrounding terrestrial environment.

Marine environments are even more variable than those of the freshwater ecosystems. More than 70% of the Earth's surface is covered by ocean. Marine environments range from depths of about 11 000 m to shallow coastal systems. Ocean temperature ranges from –2°C to as high as 36°C.

Marine environments

Marine environments are classified according to the depth at which they occur. As depth changes, so does the suite of organisms. As water becomes deeper, light is lower and photosynthetic organisms disappear. Without photosynthetic producers, there is a significant difference in the consumers present. Marine environments are divided into three regions:

- intertidal
- pelagic
- benthic

The intertidal zone is the area that is periodically covered and uncovered by the ocean. It is only found along coasts.

The **pelagic zone** is the water column of the ocean. Scientists further divide it according to the depth below the surface of the water. Each of the layers of the pelagic zone has its own unique characteristics and set of inhabitants. The phytoplankton of the upper layers of the pelagic zone are responsible for most of the world's oxygen production. They are rich in life, both microscopic and macroscopic. Light and oxygen are readily available. The temperature is fairly stable but there are small fluctuations due to changes in air temperature.

The **abyssal** pelagic zone is the layer of water found at depths greater than 4000 m. No light reaches the abyssal depths so no photosynthesis can occur there. Surprisingly, however, there is still considerable diversity of life. Species living in the deepest ocean rely on organic material sinking from the surface or the chemosynthetic bacteria that are the producers of the ecosystems surrounding the undersea volcanoes of the mid-ocean ridges.

The **benthic region** is the sea floor. The benthic region is highly variable as its characteristics are influenced to a large extent by the depth of water found above it. Near the coast, the benthic region is shallow and filled with light but in the middle of the ocean it can have more than 8000 m of water above it and is cold and dark. Apart from areas around the mid-ocean ridges, ecosystems of the deep ocean rely on nutrients filtering down from the surface or carried by currents from other parts of the ocean.

Freshwater environments

Freshwater environments are very diverse. Lakes, rivers, creeks, ponds and aquifers are just a few of the freshwater environments found on Earth. Each of the different types of fresh water has its own characteristics and its own biodiversity.

Aquifers are deep underground lakes. Queensland sits upon one of the largest aquifers in the world: the Great Artesian Basin. This huge underground lake provides water for much of western Queensland. It has its own unique ecosystem mainly consisting of micro-organisms. Where springs break the surface, small streams and lakes form and these also support a diversity of life (Figure 3.2.13).

FIGURE 3.2.13 The Great Artesian Basin underlies most of Queensland and significant parts of other Australian states.

Other aquifers, such as those found in caves across the Nullarbor Plain, support a variety of life. Many of the caves and their aquifers contain species not found anywhere else in the world. These aquifers formed hundreds of thousand, if not millions, of years ago. The populations that have inhabited the aquifers and surrounding caves have been isolated ever since, resulting in the formation of many unique species.

Aquifers are found underground but lakes are found on the surface of the Earth. Over time, lakes may change in their water volume and this can have a profound effect on the species present. Smaller lakes or ponds may even dry up entirely, resulting in most of the aquatic organisms either migrating or dying.

Rivers and creeks present different challenges to the organisms that inhabit them. Some are deep and slow-moving while others have rapidly moving water with cascades and rocks. Some waterways never dry up, while others exist for only short periods of the year.

Study of freshwater sources needs to take place year round. Rains may increase the depth, speed of movement or turbidity of freshwater sources and dry periods may have the opposite effects.

Measuring aquatic factors

While aquatic environments are affected by many of the same abiotic factors as the surrounding terrestrial environment such as temperature and rainfall, they also have a number of unique traits that need to be identified and measured.

Light, depth and turbidity

Plants and algae, which make a significant contribution to photosynthesis in many aquatic ecosystems, rely on light for photosynthesis, but light does not penetrate water very well. Some wavelengths of light penetrate water more deeply than others, and different species of plants and algae will grow at different depths (Figure 3.2.14). The depth of the water should be measured in any study of an aquatic environment. The depth of a body of water determines what producers will grow there and how abundant they will be.

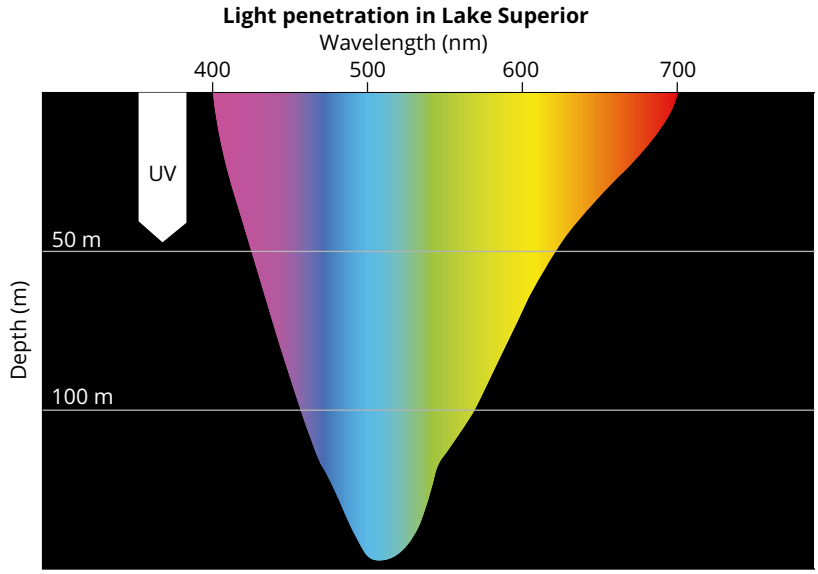


FIGURE 3.2.14 Light penetration in a freshwater lake, Lake Superior, on the border between Canada and the USA.

Light penetration is not just determined by water depth but also by turbidity. **Turbidity** is a measure of the cloudiness of the water. As water becomes more turbid, light is less able to penetrate. Turbidity is caused by particles suspended in the water. These particles may be sediments, micro-organisms, precipitated chemicals or pollutants.

Turbidity is measured in nephelometric turbidity units (NTU).

As turbidity increases, photosynthesis decreases. So does the abundance of both plants and algae, due to reduced light availability. Large plants that survive the high turbidity grow at a slower rate and become a poorer food source as sediment is deposited on leaves. This results in a loss of food sources for the herbivores which rely on the plants and algae.

A loss of herbivores has a cascade effect through the whole ecosystem. A study was made of two Queensland lakes: Lake Yumberarra and Lake Karatta. The lakes are less than 1 km apart but Lake Yumberarra is clear and Lake Karatta is turbid. It was discovered that the biodiversity and abundance of bird life on the turbid lake was significantly lower (Figure 3.2.15).

Further research showed a complete lack of plant life at Lake Karatta due to the light-reducing effects of the turbidity. This in turn resulted in there being few of the herbivorous invertebrates that supported the food chains in Lake Yumberarra.

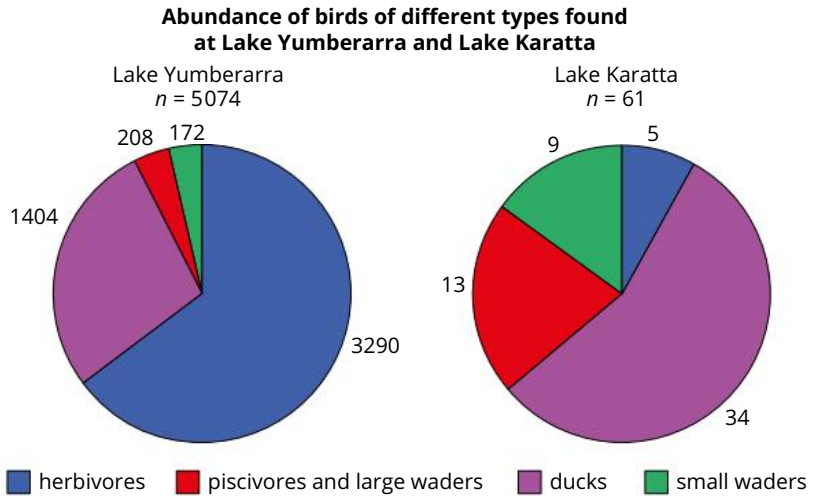


FIGURE 3.2.15 Pie charts illustrating the number and type of individual birds at Lake Yumberarra and Lake Karatta. The numbers next to each sector in the graphs are the mean number of individuals per survey across the study period.

In rivers and streams, turbidity changes along the length of the waterway. As water flow rates slow, the ability of the river or stream to carry suspended particles decreases. The water deposits the material that is causing the turbidity. While the water will become better for light penetration, the dropped sediment has another effect on the ecosystem. It fills any small spaces between gravels and pebbles that form the floor of the waterway. This changes the microhabitats available to invertebrates that inhabit the water course. A change in microhabitats results in changes in the types and numbers of organisms present.

Measuring depth and turbidity

Measuring the depth of deep water requires specialised equipment, such as sonar, but the depth of shallow water can be determined using simple tools: a long piece of string or thin rope, a tape measure and a heavy weight. Attach the weight to the string, lower the weight into the water until it reaches the bottom, mark the point on the string where it emerges from the water, pull up the weight and measure the length with the tape measure. Measurements should be taken in a variety of places over the study area.

Turbidity can be measured simply by using a **Secchi disc** (Figure 3.2.16). The disc is lowered into the water until it is no longer visible and the depth at which this occurs is noted. The greater the depth, the clearer the water.

A more accurate measurement of turbidity can be made using a turbidity tube. This is a clear plastic or glass tube with a Secchi disc at the bottom. It is 3–4 cm in diameter and around 70 cm long with graduations 1 cm apart along the side (Figure 3.2.17).

To use a turbidity tube, pour a sample of the water into the tube while looking down from the top until the disc is no long visible. Take note of the depth of water as this gives a measure of the turbidity in NTUs, as shown in Table 3.2.3.

TABLE 3.2.3 Measures of turbidity using a turbidity tube

Distance (cm)	Turbidity (NTU)
2.5	500
3.7	300
5.1	200
8.3	100
13.5	50
19	30
25	20
41.5	10
63	5

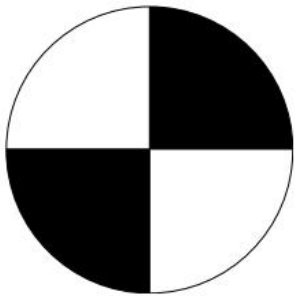


FIGURE 3.2.16 A Secchi disc is used to measure turbidity.

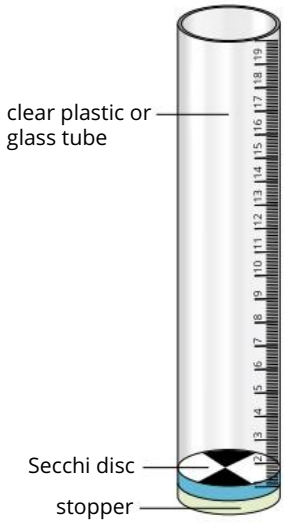


FIGURE 3.2.17 A turbidity tube.

Oxygenation, temperature and water flow

Oxygenation of the water is dependent on a number of factors including turbidity, producer abundance, temperature and flow. High levels of turbidity reduce light and therefore the abundance of producers. Oxygen is released into the water during photosynthesis, so a reduction in producers will also cause water oxygenation to reduce. The only other source of oxygen for aquatic environments is the atmosphere. Oxygen diffuses from the air into the water. This process is facilitated by turbulence, such as that seen in rapids and waterfalls.

Dissolved oxygen levels are categorised as:

- well oxygenated (> 6 mg L^{-1})
- oxygenated (> 4 and ≤ 6 mg L^{-1})
- low oxygen (> 2 and ≤ 4 mg L^{-1})
- **hypoxic** (> 0 and ≤ 2 mg L^{-1})
- anoxic (0 mg L^{-1}).

As temperature increases, the oxygen-carrying capacity of water decreases. In shallow lakes, which are most subject to the warming effects of the sun, this can have a significant effect on the biodiversity of the waterway. Sea water has a lower capacity than fresh water for holding oxygen, but temperatures are more stable; however, this may have an impact on biodiversity in shallow waters, especially in tropical regions (Figure 3.2.18).

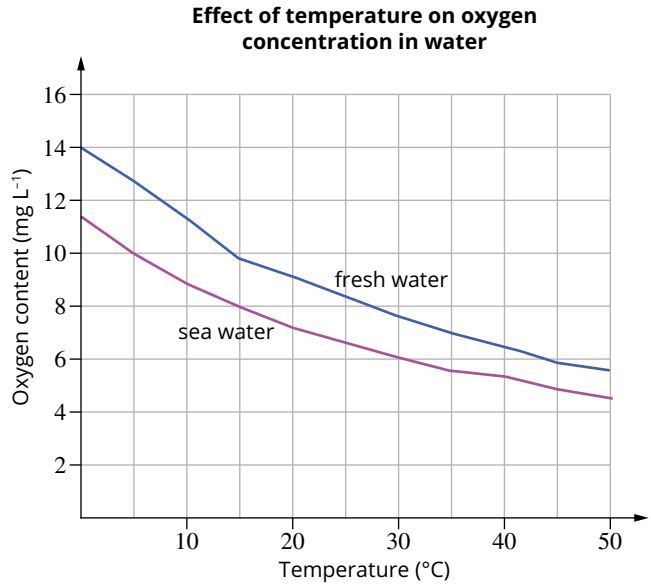


FIGURE 3.2.18 The effect of temperature on dissolved oxygen in fresh and sea water.

In freshwater environments, rivers are less affected by oxygen depletion than still waters, as turbulence causes atmospheric oxygen to mix with the water, helping to replace the oxygen used by aerobic organisms in the water. Still waters must rely on the diffusion of the oxygen from the air.

Turbidity also influences temperature. The dark particles which are suspended in turbid waters absorb more of the solar radiation falling on the water and cause the water temperature to rise. So, as well as reducing oxygen production, turbidity also reduces the oxygen-carrying capacity of the water.

Shallow lakes tend to fluctuate in temperature according to the surrounding air temperature. As the seasons change, so does the lake temperature. Large bodies of water change temperature slowly, but lakes warm up gradually through spring and summer and then cool through autumn and winter.

Deep lakes can become stratified (formation of layers) according to temperature, due to the type of circulation of the water within the lake. This stratification can have significant impacts on the concentration of oxygen in different layers (strata) of the lake. The strata are divided by a line called a thermocline. The thermocline gradually moves downwards through summer and upwards through winter, as heat is transferred by conduction at the thermocline.

This stratification is more likely to occur in temperate zones of the world rather than in the tropics, due to the large changes in ambient temperature between summer and winter (Figure 3.2.19).

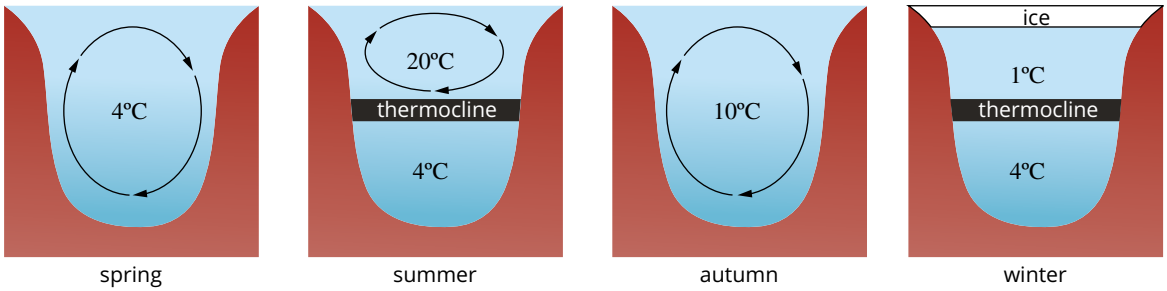


FIGURE 3.2.19 Large changes in air temperature result in a temperature stratification of deep lakes.

As well as affecting oxygen concentrations, temperature influences biodiversity because temperature influences the metabolic rate of **ectothermic** organisms, such as fish and all invertebrates. Producer metabolisms also increase. As temperature increases, so does enzyme activity and metabolism. In the short term, this can benefit animals but, if it is sustained and the water becomes oxygen-depleted, many aquatic organisms may die as hypoxic conditions are created.

The differences in tolerance to temperature change and oxygenation levels will result in differences in the species present and their abundance. Deep lakes can have quite different species living in the different layers. The amount of oxygen available for cellular respiration directly affects species growth rate, reproduction rate, life longevity and many other living processes. The lower the oxygenation of water (dissolved oxygen), the less living processes can be supported and, overall, fewer individuals can survive. The abundance of individuals, their distribution and the biodiversity of an aquatic ecosystem is significantly influenced by the amount of oxygen in the water.

Water flow in rivers and streams tends to reduce the possibility of stratification, but water courses are affected by temperature and water flow. The rate at which water moves has a number of effects on the biota of a waterway. Fast-flowing water is able to carry large amounts of sediment. It can cause fast erosion of surrounding banks and increased turbidity, but is less prone to oxygen depletion.

During times of drought, rivers and streams may stop flowing and become what are effectively a series of shallow lakes. This can create conditions which lead to rapid increases in the growth of algae (algal bloom). Algal blooms are discussed more in Module 4.3.

Measuring oxygenation, temperature and water flow

Oxygen levels and temperature are easily measured using the appropriate probes. Water flow presents more of a challenge.

The speed at which a water course is flowing is not the same as the flow rate. Flow speed can be measured by dropping a float into the water and using a stopwatch to time how long it takes for the float to reach a point at a known distance downstream.

Flow rate is a result of the cross-sectional area and the speed at which the water is flowing. Other factors also influence flow rate, and these are dependent on factors such as the rockiness of the riverbed and the steepness of the banks.

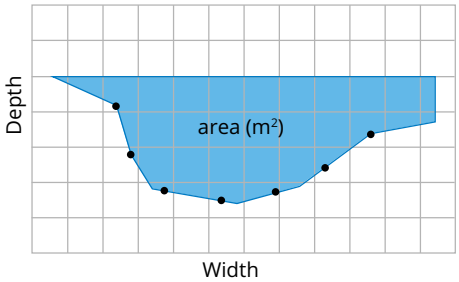


FIGURE 3.2.20 A diagram used to calculate cross-sectional area of a stream.

Flow rates are much harder to calculate than water speed. First, the cross-sectional area of the stream must be determined. This can be done by taking measurements of depth across the stream and also measuring the stream's width. This data can then be used to make a scale diagram of the stream in cross-section on graph paper. This diagram can be used to work out the cross-sectional area (Figure 3.2.20).

Flow rate can then be calculated using the formula:

flow rate (m^3/s) = cross-sectional area (m^2) × water speed ($m\,s^{-1}$) × correction factor

The correction factor allows for the differences in stream type. Correction factors are shown in Table 3.2.4.

TABLE 3.2.4 Stream correction factors

Type of stream	Flow correction factor
artificial canal with smooth sides and bed	0.85
deep slow-moving stream	0.75
small stream with a smooth bed	0.65
quick turbulent stream	0.45
very shallow, rocky stream	0.25

Results for quicker and more turbulent streams are less accurate than those for artificial canals. In line with good scientific method, all measurements should be taken several times and means used for the calculations.

pH

In all aquatic environments, especially freshwater ones, pH can be a major determinant of biodiversity. Most aquatic organisms have a defined pH tolerance within which metabolic processes are regulated by enzymes. Environments outside these pH tolerance ranges result in enzymatic denaturation and a slowing or ceasing of cellular living processes. The pH of most marine water is around 8.2, while freshwater is normally within the range of 6.5 to 8.0. Due to the smaller volumes of some freshwater bodies, fresh water can be much more variable than the ocean.

Freshwater bodies are also likely to change much more quickly due to factors such as run-off from the land. Increased levels of dissolved carbon dioxide also cause changes in pH because, as it dissolves, carbon dioxide forms carbonic acid. In the long term, if atmospheric carbon dioxide levels continue to rise, even the ocean may become more acidic. pH and salinity are also intertwined as many of the ions present in saline water are also involved in acid–base reactions. This means changes in pH may alter the availability of some mineral nutrients. Finally, acidic environments are detrimental to the formation of shells because calcium carbonate, the main constituent of shell, dissolves in acids. This is likely to significantly reduce the viability of many mollusc populations.

Like the terrestrial environment, salinity and pH of seas and waterways are easily measured using appropriate probes. Measurements should be collected over time as factors such as rainfall or drought can mean that both salinity and pH can vary significantly over time, especially in some coastal environments

CASE STUDY 3.2.1

Abiotic influences on abundance of the green sea turtle on Raine Island

Raine Island is a part of the Queensland's Raine Island National Park, situated at the northern end of the Great Barrier Reef. The park also contains two other land masses, Moulter Cay and MacLennen Cay. Of the three, Raine Island is the most biologically diverse.

Raine Island is one of the largest and most ancient green sea turtle rookeries in the world. During nesting season, up to 60 000 female turtles lay their eggs in the sand along the beaches of Raine Island. This has been happening for at least one thousand years. Reproductive success at Raine Island has a large influence on the abundance of green sea turtles in the western parts of the Pacific Ocean.

Turtles are air-breathing reptiles, so they must lay their eggs on land. Females only reproduce every 2 to 6 years, depending on how well they have built up fat stores, so the number of nesting females varies from year to year.

Green sea turtles are a species under threat. Habitat loss, boat strikes, rising sea levels and changes in beach structure of some islands are all causing a reduction in turtle populations. Raine Island is important in the fight to save the green sea turtle from extinction.

Unfortunately, in recent years reduced sand depth, along with erosion causing the formation of extra rocky areas, sand cliffs and reducing beach size, has impacted on the turtle population. The female green sea turtles crawl up onto the sand and dig out a nest and lay their eggs. They generally return to the beach where they hatched to lay their eggs and they nest in the same place each time. As the beach erodes, overcrowding becomes an issue.

A reduction in hatchings occurs periodically as a result of the inundation of nests by sea water during high tides or storms. Eggs are washed away or the embryos asphyxiate (die from lack of air). Embryonic turtles rely on diffusion of oxygen into the egg from the surrounding environment to sustain respiration. The diffusion of oxygen happens at a slower rate in water, so over a period of time the embryos begin to die. Figure 3.2.21 shows the results of seawater flooding on hatching success. Results shown are for all eggs in the nest and for eggs that were observed to contain living embryos before the inundation occurred.

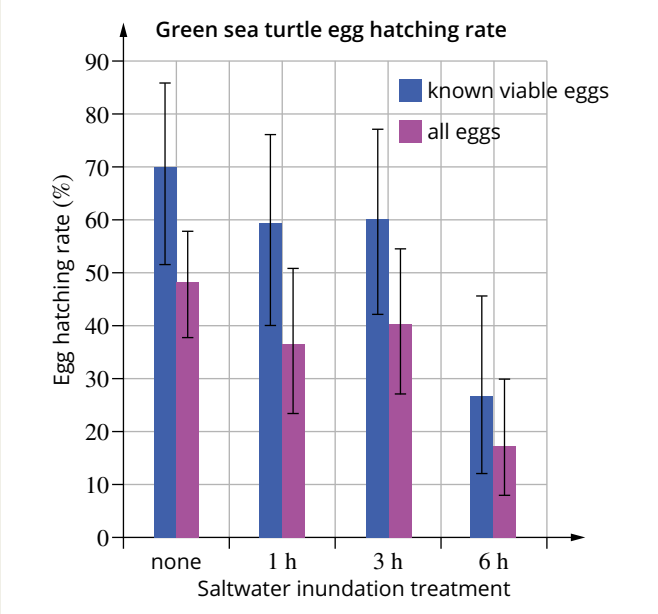


FIGURE 3.2.21 The hatching success of green sea turtles when nests are flooded with sea water compared to no inundation.

During periods of overcrowding, nests are more likely to be dug in areas which have a higher chance of being flooded due to competition for breeding space. Another consequence of this fight for space is that many nests are dug up and eggs are damaged by females who arrive later as they try to dig nests for their own eggs. Apart from loss through direct damage, turtle embryos also die due to oxygen depletion in the soil. This depletion is thought to be due to increased bacterial activity, which damages eggs. A number of factors, both biotic and abiotic, are responsible for the reduction in hatching success.

Many adult females die as they return to the sea after laying their eggs as a result of rocky areas formed by wave action and erosion. The female turtles fall down the small 'cliffs' which form and either become stuck or are flipped onto their backs (Figure 3.2.22 on page 108). These turtles eventually die of dehydration or exhaustion as they are unable to turn themselves over. Fencing has been placed in front of these cliffs to stop turtles falling over them.

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FIGURE 3.2.22 Rocky outcrops formed as a result of erosion create small cliffs and crevasses which can flip turtles over or trap them on their edges when they are returning to the sea.

In September 2014, a project was undertaken to reprofile the beach by increasing the height of a section of the beach. Sand was piled on the beach. The reprofiling increased the beach area as well as its height, reducing the chances of nests being dug up or inundated. It was hoped that a simple change in the abiotic environment would increase the breeding success of the green sea turtles and reduce the chance of their extinction. In recent years, hatching success on the island has increased from 36% to 56% and clutch destruction in the nests has been reduced from 46% to 28%.

Another problematic abiotic factor thought to have an important impact on green sea turtle abundance is temperature. Like many reptiles, sex determination of embryo green sea turtles is largely determined by nest temperature at certain critical developmental points. The pivotal temperature, which results in equal males and females, is 29.3°C. Temperatures above this produce more females and temperatures below this produce more males. The further the temperature is from the pivotal, the greater the sex imbalance. Figure 3.2.23 shows the variation from the pivotal temperature of nests at Raine Island from 1960 to 2015.

Examination of the data suggests that as the current group of sub-adults and juveniles reach maturity there will be a serious sex imbalance, with many females and few males. A severe sex imbalance could lead to the collapse of the turtle population.

Review

1. Describe the trends in the graph in Figure 3.2.21.
2. Conclude the results shown in the graph in Figure 3.2.21.
3. Determine the length of time green sea turtle nests can be underwater before there is a significant decrease in hatching success. Explain your answer.
4. Propose how the reprofiling of the beaches of Raine Island could affect the abundance of green sea turtles in the future.
5. Argue whether or not a sex imbalance could result in the collapse of the entire worldwide green sea turtle population in the future.

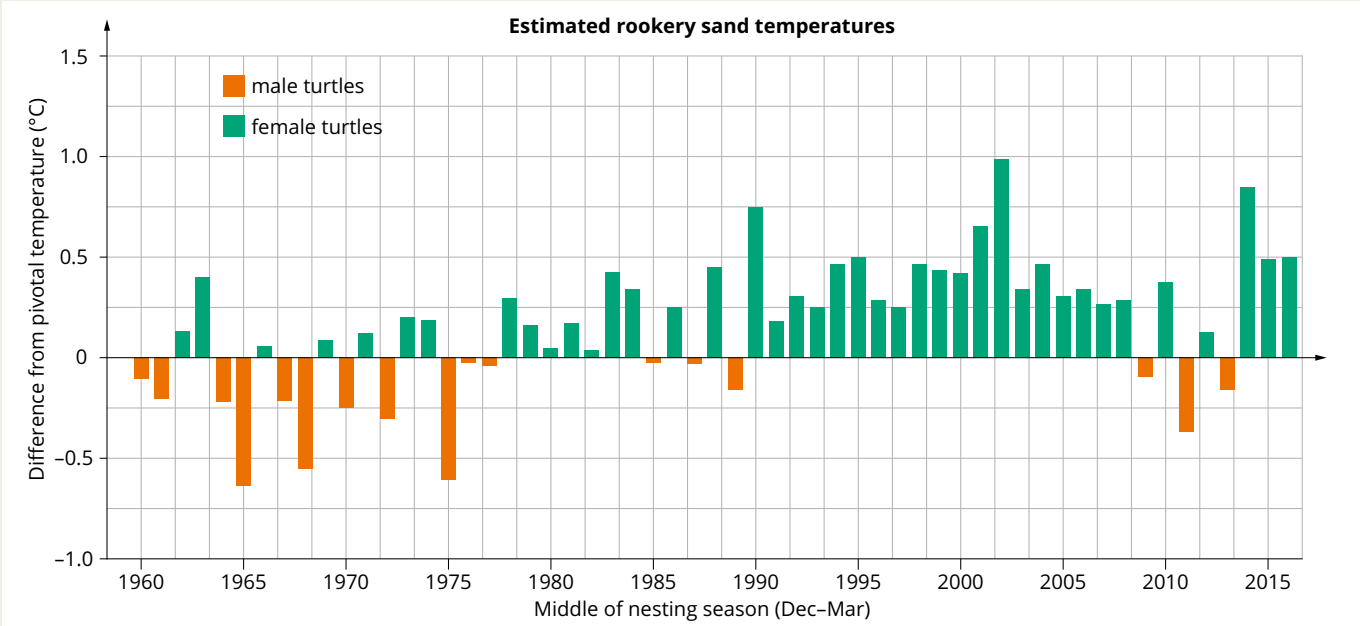

FIGURE 3.2.23 Nest temperature variation at Raine Island. Regular recent variations above the pivotal temperature are likely to lead to a serious sex imbalance in the future.

Ecosystems variability

Ecosystems are variable. Variations in geography, climate and time all impact on ecosystems. Ecosystems change over time. The biodiversity of the Daintree will be different in the wet season from the dry season. Some animals stay only during the dry season, while others are only there during the wet season. Migratory birds come and go. Vegetation is also different through the year as some plants germinate and grow in a very short period of time. This is obvious in places like Kati Thanda–Lake Eyre, where for most of the time the ecosystem is a vast, arid salt plain inhabited by a few hardy species, but on the rare occasions when rain falls, the area teems with a large variety of species.

Other ecosystems are variable because they are spread over a large area. The Great Barrier Reef extends from around 8°S to approximately 24°S. Any ecosystem this large will contain many different habitats and each of those habitats will support its own unique mix of species.

Ecosystems also vary through long periods of time in a process called succession. In this process, one ecosystem replaces another until a final stable ecosystem, which lasts for a long period of time, is formed. The processes of succession will be explored further in Module 4.4.

CASE STUDY 3.2.2

The Great Barrier Reef

The Great Barrier Reef is around 2300 km long and covers an area in excess of 344 400 km^2. Most of it is a part of the Great Barrier Reef Marine Park, which starts at the tip of Cape York and extends to just north of Bundaberg. It ranges in width from 60 to 250 km. The depth of the water varies from an average of 35 m in the areas close to the shore to greater than 2000 m on the outer areas of the reef where the continental shelf ends. It is formed from three sorts of reef structure: fringe reefs, which form in shallow waters near the coast; atolls, which are rings of coral that formed around now sunken islands; and barrier reefs, which are structurally much stronger than either of the other two kinds.

The Great Barrier Reef is a biodiversity hotspot. Apart from many species of sponges, molluscs, algae and cnidarians (corals, sea pens, sea jellies), there are also around 1625 species of fish and a number of iconic species such as dugongs, dolphins and sea turtles inhabiting the reef and its surrounds.

The corals of the reef are divided into two main groups: soft corals, belonging to the subclass Octocorallia, and hard corals, belonging to the subclass Hexacorallia. The soft corals do not produce the hard calcareous exoskeletons which form reefs but they do add to reef biodiversity.

Different areas of the reef play host to different genera of soft corals (Figure 3.2.24a) along with many hard or stony corals such as brain coral (Figure 3.2.24b). It is the stony corals that build reefs.

Members of the genus *Dendronephthya* are common in deep water. Coastal areas affected by periods of varying salinity play host to *Sinularia flexibilis* and turbid areas lack members of the family Nephtheidae.

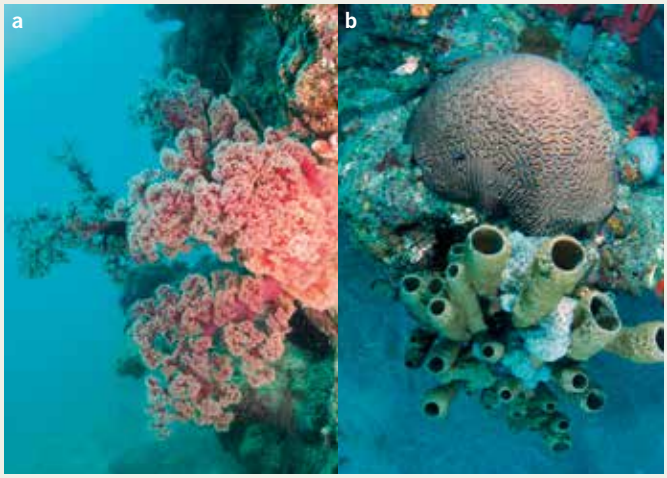

FIGURE 3.2.24 The Great Barrier Reef features (a) soft coral and (b) stony coral.

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In fact, as Figure 3.2.25 shows, there is great diversity in the species richness of the Octocorallia across the Great Barrier Reef.

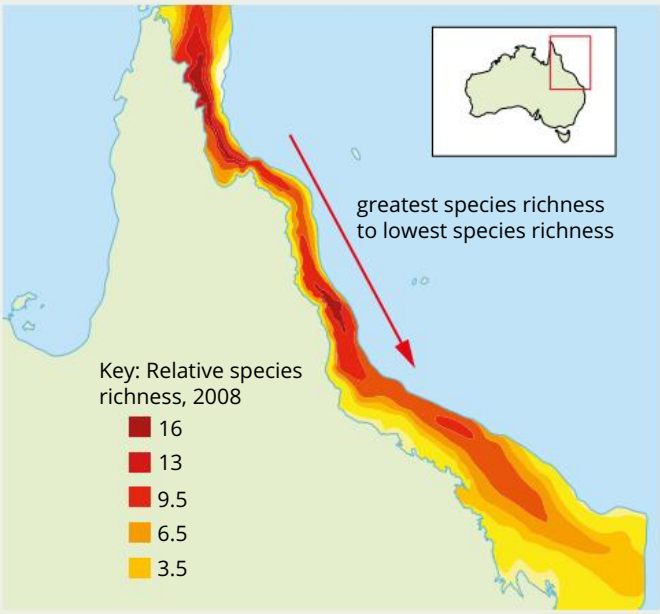
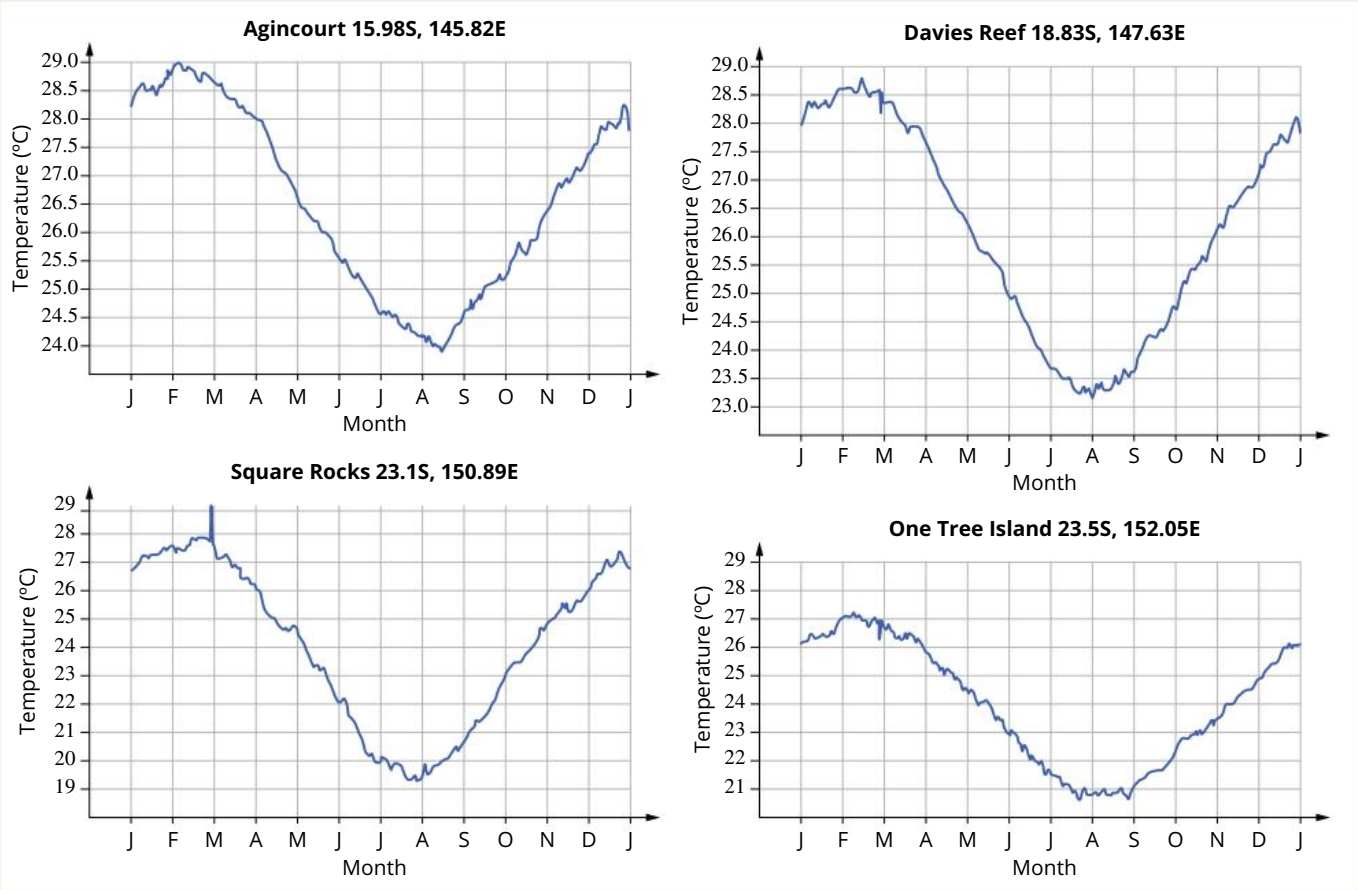


FIGURE 3.2.25 Spatial distribution of octocoral richness across the Great Barrier Reef.

Corals live in a type of symbiotic relationship called mutualism with algae called zooxanthellae. The zooxanthellae are also responsible for the colours of the corals. The algae live in the tissues of the coral polyps and provide the corals with oxygen and sugars. The algae gain a safe place to live and are provided with some of the raw materials of photosynthesis. The zooxanthellae associated with corals are photosynthetic and so are limited in their ranges according to light intensity. Light intensities vary with water depth across the reef. Most corals are found in shallow waters but studies of the Great Barrier Reef have found members of the genus *Leptoseris*, a hard coral, living at a depth of 125 m. Sponges and sea fans are also found in deep water, as they are not light-dependent. At these depths, the biodiversity is much lower than in shallower waters as few photosynthetic organisms can survive in the low light intensities which prevail there.

The large size of the Great Barrier Reef means that different areas will experience different conditions. Water temperatures vary across the reef (Figure 3.2.26).

FIGURE 3.2.26 Mean water temperatures throughout the year at various points along the Great Barrier Reef.



Most corals are very temperature-sensitive, with different species having different tolerance ranges. When the coral polyps are subjected to heat stress they eject their algal partners and the corals lose their colour. This is called a bleaching event. The coral polyps can only survive for a limited time without their symbiotes. Bleaching events reduce biodiversity as the algae are ejected and many of the corals die. Different species have different tolerances to temperature, so bleaching does not affect all species equally. In 2016, a major bleaching event occurred across the reef but not all areas were equally affected (Figure 3.2.27). More southern and deeper waters were less affected.

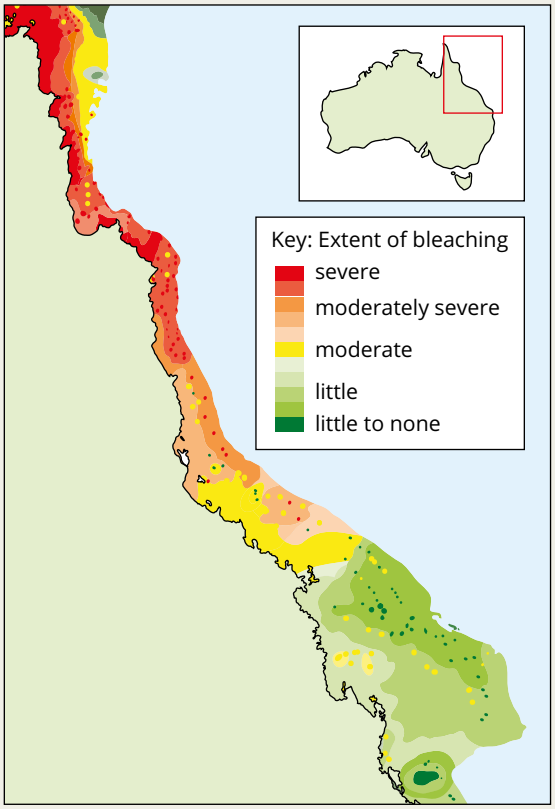


FIGURE 3.2.27 The extent of bleaching in March 2016. Extent of bleaching ranges from little to none (green) to moderate (yellow), moderately severe (orange) and severe (red).

Coral bleaching is a threat to the long-term survival of the reef. Another threat is the crown-of-thorns sea star (*Acanthaster planci*). Periodically, the sea star increases in dominance along the reef as it goes through population 'boom and bust' cycles.

During the wet season, floodwaters wash nutrients into the coastal waters. This provides food for algae, which greatly increase in number. Both nutrient levels and sediment loads increase as a result of human activities such as land clearing, which increases erosion, and the use of fertilisers, which increase nutrient levels in the rivers that flow into the waters surrounding the reef. The resulting increases in algae provide more food for the algae feeders of the reef, including the larvae of crown-of-thorns sea star. Unfortunately, the adult sea star does not feed on algae. It feeds voraciously on coral polyps. The sea stars leave only the skeletons of the corals behind (Figure 3.2.28). Unlike bleached corals, which are still alive but have lost their colour, these corals are dead.

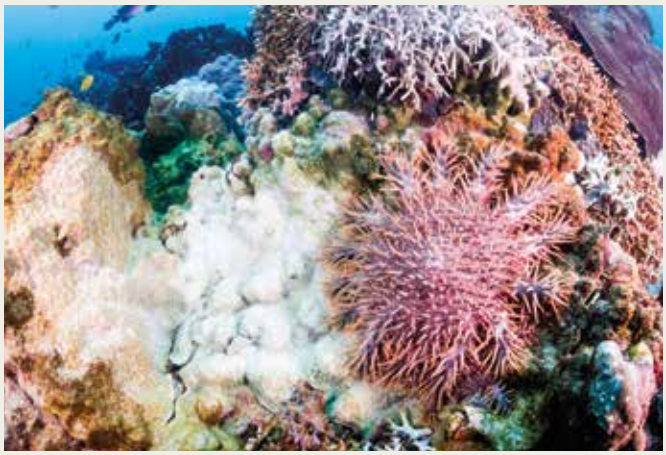


FIGURE 3.2.28 A crown-of-thorns sea star leaving bleached, dead coral behind after feeding.

In areas of moderate coral coverage (40–50%), crown-of-thorns sea stars at densities greater than 15 per hectare can eat the coral polyps at a faster rate than the coral can grow.

The periodic increase in the sea star population has had a number of effects on the biodiversity of the reef. Four outbreaks of crown-of-thorns sea stars have occurred during the last few decades (1961–68, 1978–91, 1993–2005 and 2011–?). Scientists have fossil evidence that periodic plagues have occurred for as long as 8000 years, but evidence suggests that the plagues are becoming more regular, reducing the ability of the reef to recover.

Initially the predation reduces the percentage cover by corals, and rare species become much rarer or locally extinct. As the reef recovers, fast-growing coral species are advantaged over slow-growing species. Algae, which are even faster growing, increase their coverage. Higher abundance of algae advantages fish species that feed on algae, so these increase in abundance while coral-feeding fish decrease due to increased competition for the remaining food. In this way, regular crown-of-thorns sea star plagues can change the biodiversity of the reef.

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CASE STUDY 3.2.2

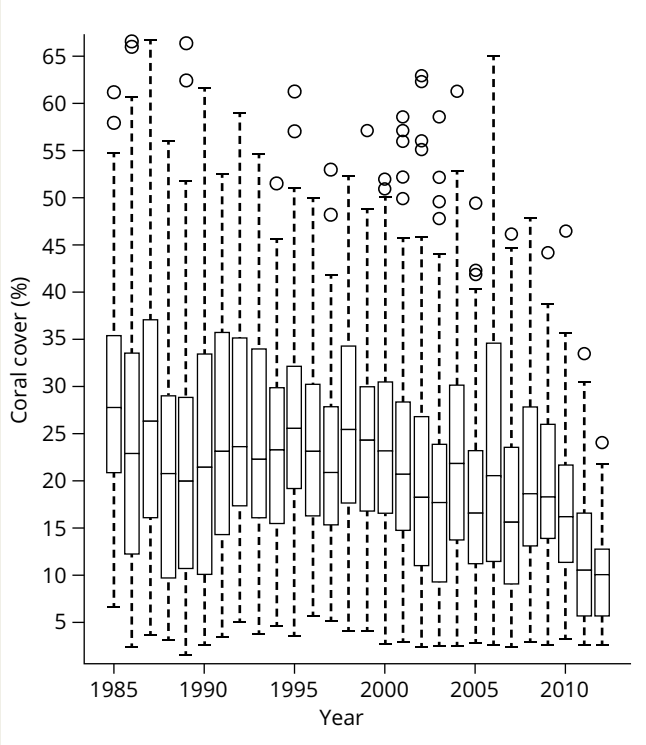

FIGURE 3.2.29 Box plots of percentage coverage of corals across the Great Barrier Reef over 27 years shows a substantial decline.

Regular surveying of the reef since 1985 has shown a 50% reduction in coral coverage. Tropical cyclones are believed to be responsible for 48% of that decline, bleaching due to temperature causes 10% of the loss and 42% is attributed by scientists to the depredations of the crown-of-thorns sea star. Figure 3.2.29 shows the decline in coral percentage cover over the period 1985–2012. The box plots show the 25%, 50% and 75% percentiles of the distribution of corals across the reef.

Review

1. Compare the median percentage cover of coral in 1985 to that in 2012. Use Figure 3.2.29.
2. Describe how the species richness of the octocorals varies throughout the Great Barrier Reef.
3. Propose how human activities may have caused the increase in crown-of-thorns sea star plagues.
4. Name the relationship that exists between corals and zooxanthellae.

WS 3.1.6

CASE STUDY 3.2.3

An intermittent lake of the interior —Lake Yumberarra

Lake Yumberarra is a small freshwater lake situated in the Currawinya National Park in south-western Queensland. It is intermittent in nature. At times it is full of water but on other occasions it dries out completely. During the drying period, as the water in the lake decreases it becomes increasingly brackish. In 1995, the lake filled with water. The lake maintained at least some water until 2003 but there were periods when levels dropped during that period and water quality deteriorated. For a short time, the lake was completely dry.

Species surveys of the lake showed considerable changes in both species richness and species abundance when the lake contained water. Figure 3.2.30 shows the changes in species richness and waterbird abundance during the period 1998–2002.

These changes reflected the changes in water depth and quality in the lake. Invertebrate species varied with water pH and salinity. Some species were more tolerant than others to changes in both of these factors. Inevitably, as invertebrate numbers fell, so did the richness of other species, most notably the waterbirds that were attracted to the lake. Figure 3.2.31 gives a snapshot of conditions at the lake over the 16-month period from September 1998 to December 1999. Note that the water levels are measured from when the lake was full. Negative values represent the lowering of water levels in the lake.

The lake was completely dried out between December 1997 and April 1998, when it refilled. By 2002, the lake was drying out and by early 2003 it was completely dry and did not fill again within the study period.

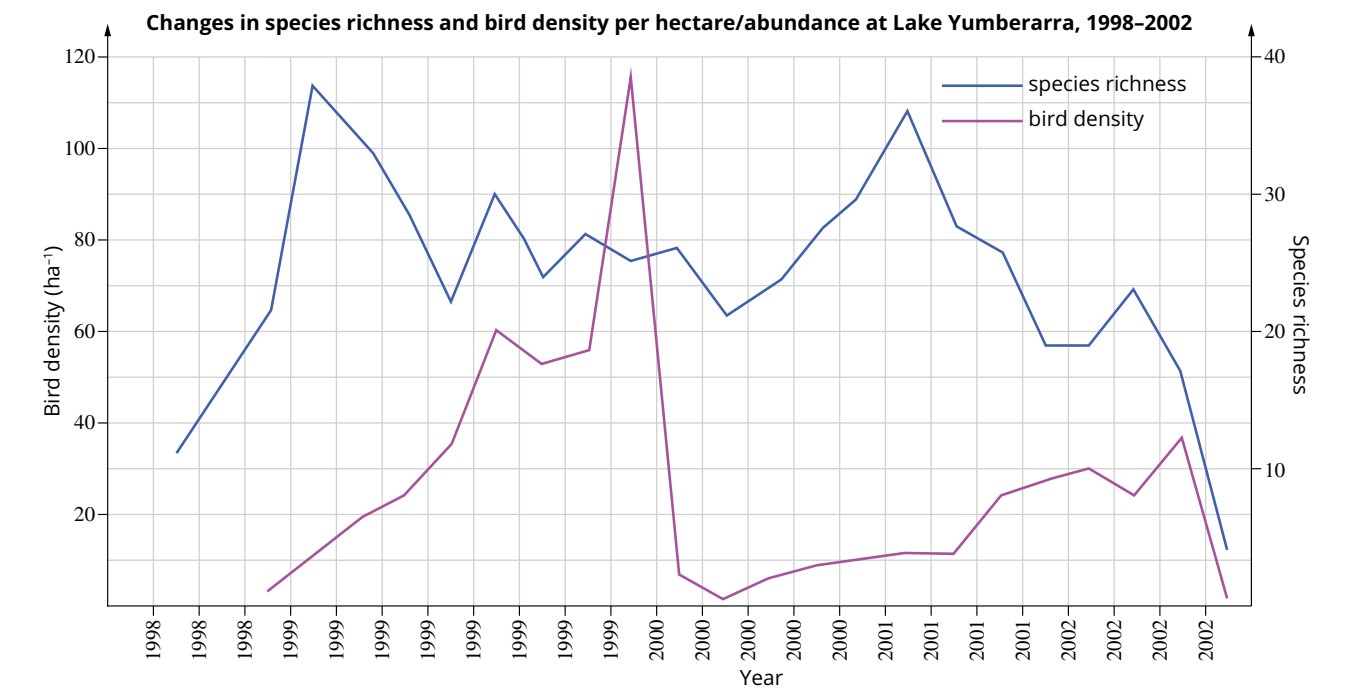

FIGURE 3.2.30 Changes in species richness (blue line) and bird density per hectare/abundance (purple line).

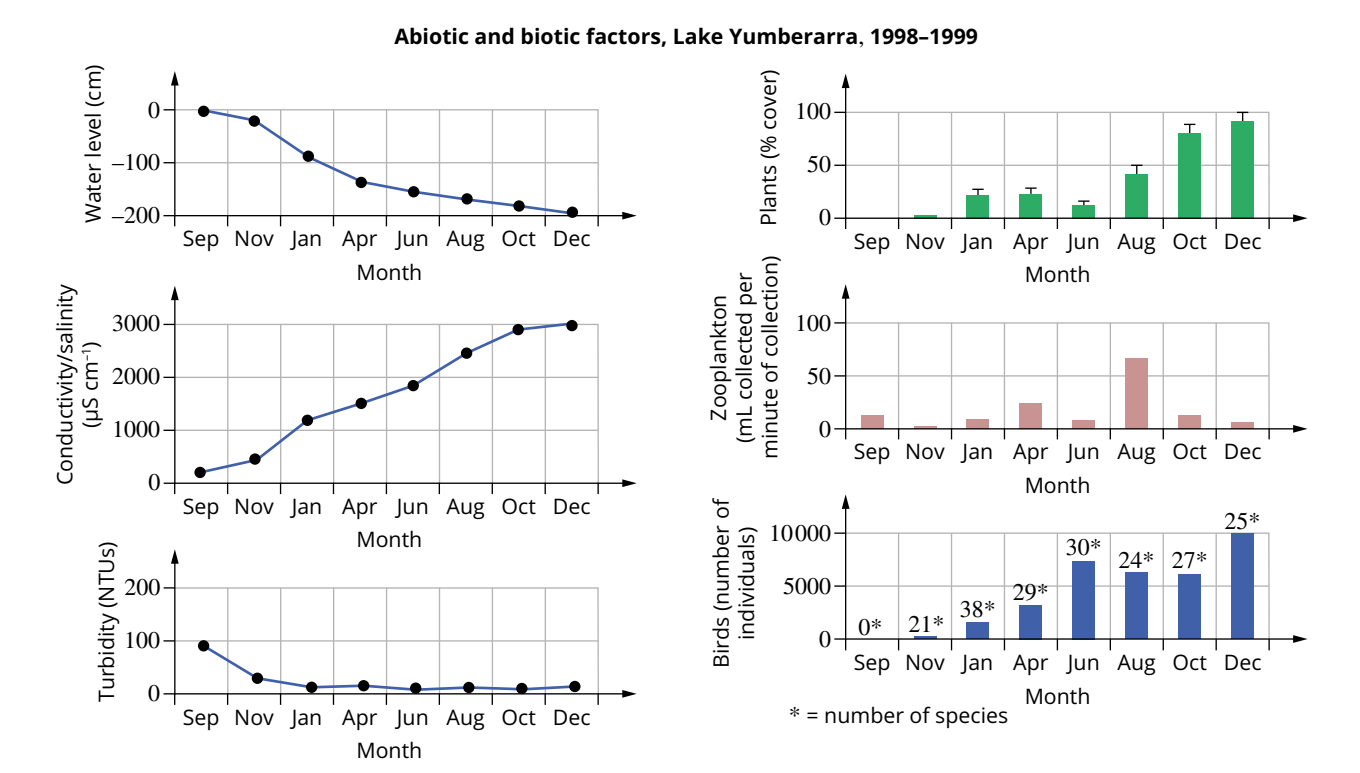

FIGURE 3.2.31 Changes in various biotic and abiotic factors at Lake Yumberarra from September 1998 to December 1999.

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CASE STUDY 3.2.3

FIGURE 3.2.32 The number and type of individuals increases dramatically after rain in the region of Kati Thanda–Lake Eyre from (a) no obvious animals to (b) a significantly large abundance.

Lake Yumberarra is typical of the intermittent waterways of the arid interior. All go through 'boom and bust' cycles of species abundance and diversity determined by the presence or absence of water. Species that live in this highly changeable environment either have highly specialised traits to cope with the changes or, like many waterbirds, are migratory, moving from one water source to another.

Figure 3.2.32 shows the effect of these cycles of rising and falling abundance and diversity at another intermittent lake of the interior, Kati Thanda–Lake Eyre. Waterbird numbers when the lake is filled have been estimated to exceed 100 000 as migratory birds arrive to take advantage of the increase in food.

Like at Lake Yumberarra, the food source for these birds is the millions of invertebrates that reproduce as soon as water becomes available, and fish washed down the river channels, which breed rapidly and also feed on the many invertebrates and algae found in the floodwater.

Review

1. Propose the reason for the low species richness in 1998 at Lake Yumberarra.
2. Summarise the changes in biotic and abiotic factors that occurred at Lake Yumberarra through the study period and argue their interrelationships.

CASE STUDY 3.2.4

Algal succession on a coral reef

Making sense of the influences of species interactions and how ecosystems develop and change over time is complicated and requires masses of data, so most studies focus on a single aspect within a single ecosystem. These different studies can then be pooled to draw conclusions.

One study undertaken in the mid-1990s looked at the succession of algal species colonising rocks on a coral reef in Hawaii. The study examined the effects of one species of damselfish on the growth and diversity of algae on newly formed rock. Damselfish are herbivorous and feed on algae within their territory. They are highly territorial and protect their territories aggressively from all other fish species.

In order to investigate the process of succession and the development of a stable ecosystem, 144 plates of rock were placed on the sea floor. Each plate had an area of 50 cm^2. Plates were distributed evenly between three areas: within the damselfish territories, outside the damselfish territories, and inside cages. Cages excluded all fish species, including the damselfish. The outside plates allowed the scientists to identify the effects of all fish on the algal succession, while the caged plates showed how succession would proceed in the absence of all fish. The plates in the caged and outside areas acted as controls.

Data was collected for 350 days after the placement of the plates. Algal species diversity, richness and evenness were all calculated and, along with the biomass of algae in each area, were determined regularly throughout the study period. The study investigated how a single species, the damselfish, could alter the progress and direction of algal succession. Algal biomass was measured throughout the study period. The results are shown in Figure 3.2.33.

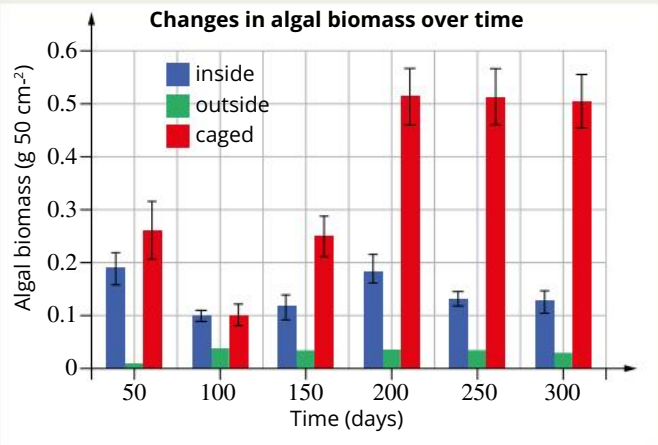

FIGURE 3.2.33 Key data points for algal biomass inside damselfish territories, outside damselfish territories, and within cages that excluded all fish. Error bars indicate ± 1 standard error (SE).

Algal biomass data for the three study areas followed an expected pattern. Biomass increased in the caged areas where all herbivorous fish were excluded. In uncaged areas outside the damselfish territories, biomass remained fairly stable after an initial small increase. This was expected, as many species of fish rely on algae as their main food source. In the territories maintained by the damselfish, biomass initially increased and then showed a small decline. The damselfish stop other fish species grazing on the algae in their territory, allowing the algae to increase in abundance, but because the damselfish feed on the algae, it was lower in biomass than in caged areas.

As algae are the primary producers of marine ecosystems, differences in their richness, diversity and abundance will affect the other species through changes in the food webs. After the plates were placed (day 0), they were rapidly colonised by algal species but in the outside areas, fewer species became established. Species richness diminished over the next 75 days in the caged areas. This was similar in the outside areas, but species richness was maintained in the damselfish territories. From around day 75, species richness was similar in caged and outside areas, but in the areas protected by the damselfish species, richness was higher. A single species is influencing the ecosystem to increase species richness within its territories (Figure 3.2.34).

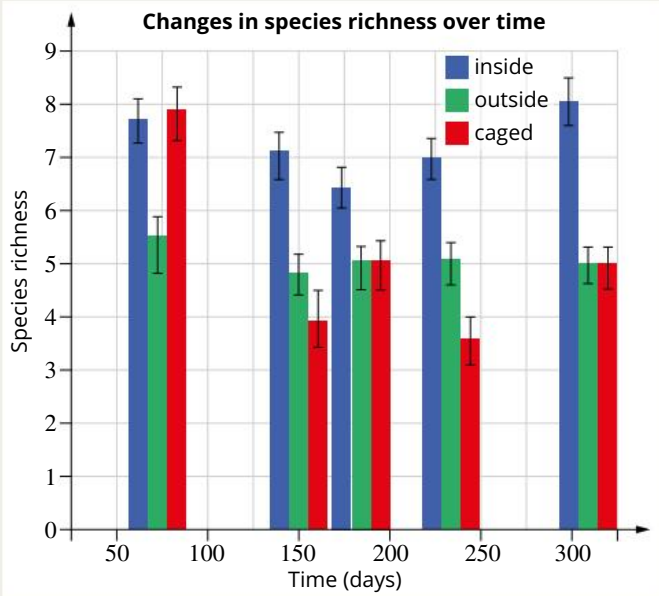

FIGURE 3.2.34 Key data points, with error bars, for algal species richness inside damselfish territories, outside damselfish territories, and within cages that excluded all fish. Error bars indicate ± 1 SE.

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It is clear from the data that herbivorous fish species also influence species evenness. In damselfish territories and in the outside areas, species evenness is greater than in the caged areas and remains consistently so over the entire study period. The absence of fish herbivory allows some algal species to become dominant and reduces the abundance of others (Figure 3.2.35).

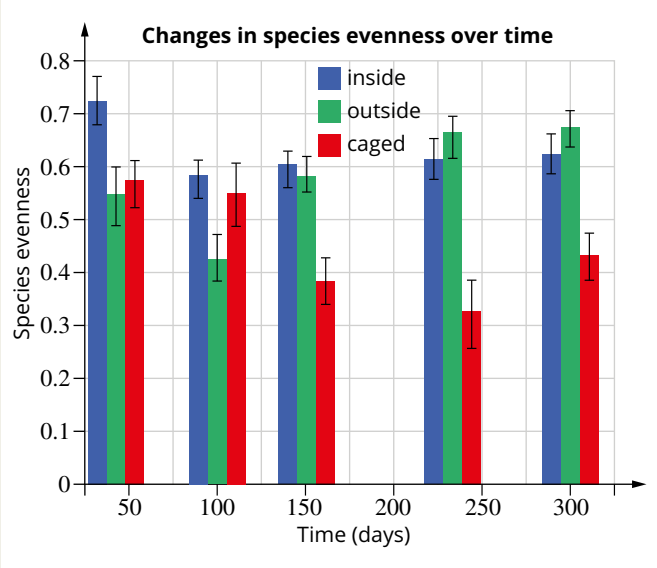

FIGURE 3.2.35 Key data points for algal species evenness in damselfish territories, outside damselfish territories, and within cages that excluded all fish. Error bars indicate ± 1 SE.

Herbivorous fish also influence species diversity and how it changes over time, as can be concluded from the data shown in Figure 3.2.36.

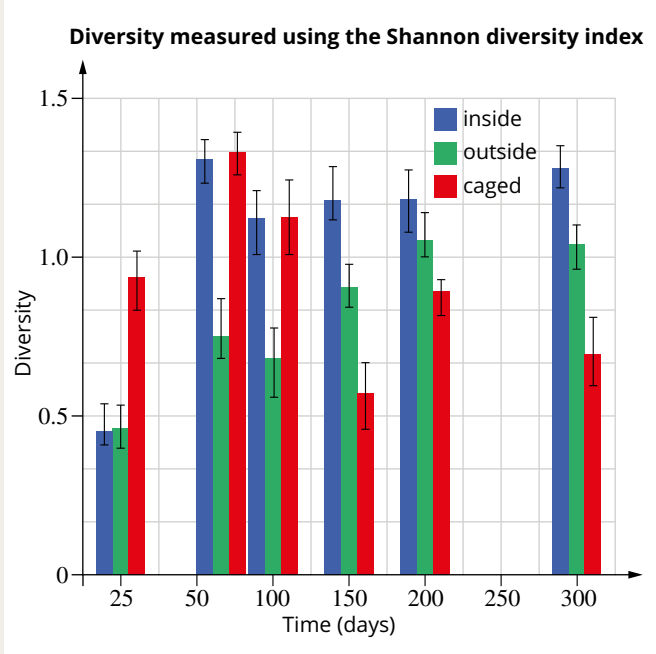

FIGURE 3.2.36 Key data points for algal species diversity using the Shannon diversity index (larger numbers indicate higher diversity). Error bars indicate ± 1 SE.

In this study, the influence of fish herbivory on the succession of one element of a marine ecosystem, algal succession, was investigated. Even in this very limited study, which took place over a short period of time, there were several influences on biodiversity, including types of producers, types of consumers and consumer behaviours. Within wild ecosystems, there would be many other variables affecting the ultimate outcome. However, the study does allow us to conclude that interactions between species do influence the speed and direction of change within ecosystems.

Review

1. Describe the changes in species diversity over the period of the study.
2. Conclude the effect of damselfish on the species richness in this study and propose an explanation.
3. Compare the amount and species of algal consumption between damselfish and all fish.
4. Draw conclusions about the influence of damselfish on the health of the ecosystem within the study areas.

SCIENCE AS A HUMAN ENDEAVOUR

The Convention on International Trade in Endangered Species of Wild Fauna and Flora

Habitat loss, climate change and over-exploitation of resources all offer challenges to the maintenance of biodiversity. Each of these issues has been addressed by international agreements with varying degrees of success. One such agreement is the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

CITES is an international agreement that places obligations on signatories to regulate the trade in any species listed on its register. Approximately 30 000 species of plants and 5800 animal species have at least some level of protection under CITES.

The agreement was first proposed in 1963 at a meeting of the World Conservation Union (IUCN), but it was not until 1975 that it was implemented with 80 countries as signatories. Since that time, there has been a steady increase in membership, as more governments have come to recognise the importance of maintaining the Earth's biodiversity. As of 2015, there were 183 signatories representing the vast majority of the world's countries.

As more countries have signed CITES, the majority of international trade in endangered species has become regulated. Most trade now occurs between captive-bred populations, protecting individual organisms still in the wild. Prior to CITES, all trade in wildlife was unrestricted and unmonitored. It was impossible to tell if plants or animals were being driven to extinction. Under CITES, trade in most threatened species is banned under all but exceptional circumstances. In addition, trade in other plants and animals on the register is carefully monitored.

Any person wishing to trade in a listed organism must obtain a licence and register the trade. This allows the committee and governments to decide to increase the level of protection for a species if it is being over-exploited. Since the initial signing of the agreement, the number of registered trades has increased steadily. This does not mean that there is more trade in endangered species, just that the trade that is occurring is more closely monitored. The growth in trade licences registered through CITES is shown in Figure 3.2.37.

While not without its issues, CITES has reduced the trade in endangered species. The CITES ban on trade in elephant products, primarily ivory, came into effect in 1989. In 1997 and 2008, one-off sales of ivory from stockpiles held by African governments to China and Japan were permitted. Many people argued against these sales because they claimed that they would drive poaching and result in harm to elephant populations.

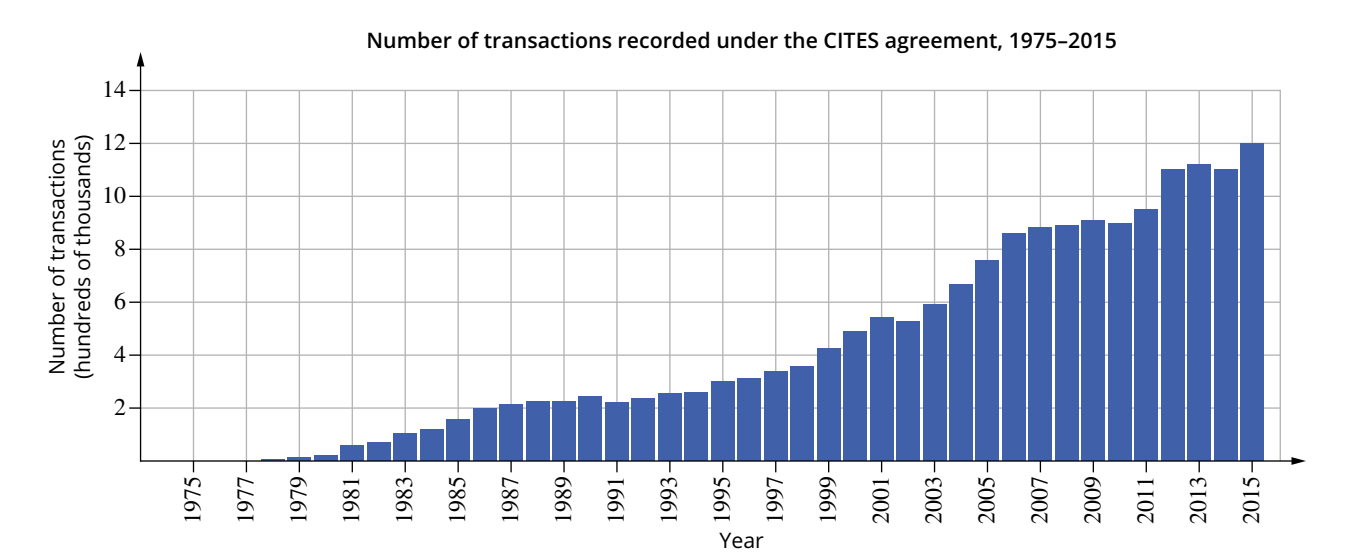

FIGURE 3.2.37 The increase in trade regulated by the CITES agreement since 1975.

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Studies of African elephant population sizes have taken place over the period after the implementation of the elephant trade ban. Figure 3.2.38 shows the African elephant population trends from 1995 to 2014.

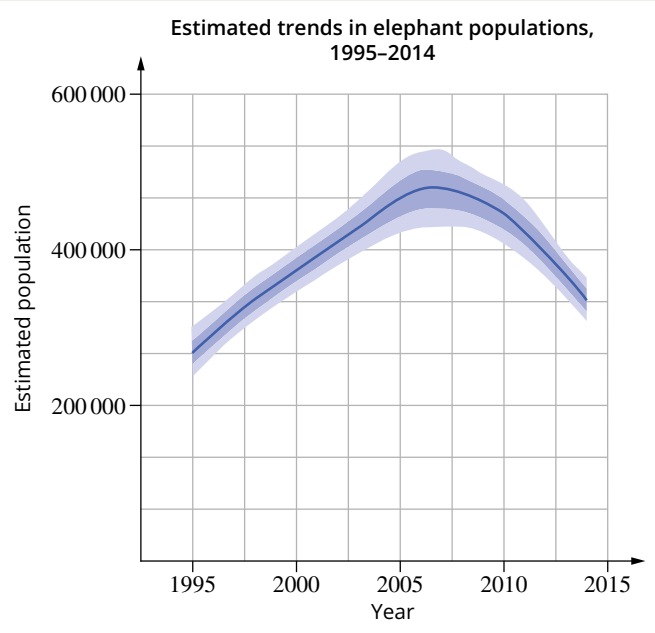


FIGURE 3.2.38 Estimated trends in elephant populations during the period 1995 to 2014. Dark shaded area indicates ±1 standard deviation and light shaded area indicates 95% confidence interval.

During 2014–15, the Great Elephant Census was undertaken across Africa to find out the remaining population of savanna elephants. Aeroplanes flew thousands of kilometres of transects across elephant habitat to count the elephant populations. The results of that survey by country are shown in Table 3.2.5.

One of the largest remaining legal markets for ivory was China, until it was banned in 2016. Ivory trade will be banned in Hong Kong in 2021. In China now, and in Hong Kong by 2021, severe penalties for selling ivory will be instituted. Conservationists hope that this will stem the tide of poaching, which is causing the decline in elephant populations. If the many opponents to legal trade in ivory are correct in the belief that it is the demand of legal trading that drives the killing of elephants for ivory, then elephant populations may again begin to increase. Unfortunately, some current populations may be too small to recover.

Review

1. Discuss whether any evidence has been presented to support the contention that permitting the sale of ivory-collected stockpiles by governments is driving the poaching of elephants.
2. Explain which populations would be most under threat even if all poaching stopped tomorrow.

TABLE 3.2.5 Estimated elephant populations across Africa, 2014–15

Country	Estimated elephant population	Country	Estimated elephant population
Angola	3395	Mali	253
Botswana	130 451	Mozambique	9605
Cameroon	148	South Africa	17 433
Chad	743	Tanzania	42 871
Democratic Republic of Congo	1959	Uganda	4864
Ethiopia	799	West Africa	8911
Kenya	25 959	Zambia	21 759
Malawi	817	Zimbabwe	82 304

3.2 Review

SUMMARY

- Climate is a major influence on the biodiversity of ecosystems.
- Soils of terrestrial ecosystems determine the types of plants which grow and, in turn, this determines the types of animals present.
- Characteristics that vary within soils include pH, porosity, structure, salinity, nutrient content and depth.
- Loss of genetic diversity can lead to extinction because species are less able to adapt to environmental change.
- Aquatic environments include both freshwater and marine.
- In water, salinity, turbidity and water depth all influence the richness and abundance of species present.
- Characteristics of all environments can be measured using appropriate methods and equipment.
- Ecosystems are dynamic in their nature. They vary through time and across their geographical range.

KEY QUESTIONS

Retrieval

1 List the major determinants of soil quality in Australia.

2 Identify the largest cause of reduction in percentage cover of the Great Barrier Reef.

3 Explain why the biodiversity of cold climates tends to be lower than that of warm climates.

Comprehension

4 Distinguish between benthic and abyssal.

5 Sundews are frequently observed growing in leached soils. Explain this distribution.

6 Explain how soil pH influences the biodiversity of an ecosystem.

7 Explain why many species of plants die in soils that are waterlogged.

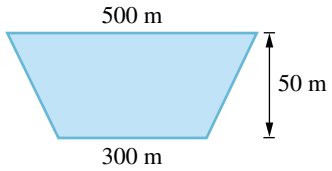
8 Estimate the turbidity of a waterway for which the Secchi disc became invisible at 10 cm of water in a turbidity tube.

9 Explain how variations in water depth across the Great Barrier Reef affect the biodiversity of the ecosystem.

10 The trees in an orchard are showing signs of nutritional deficiency. The pH of the soil is found to be 4.2. Explain why the plants are showing signs of disease.

Analysis

11 Water flows down an artificial channel shaped as shown. The water is flowing at 1.3 m/s.



Calculate the flow rate. Use the formula for the area (A) of a trapezium:

$$A = \frac{1}{2}(a + b)h$$

where
a and b are the parallel sides
h is the distance between them.

12 A soil is 25% sand, 50% silt and 25% clay. Determine the soil type.

13 Explain how the data graphed in Figure 3.2.12 (page 99) answers the research question, 'Does area size influence species richness?'

MP 2

Comparing species diversity of communities

Research and planning

Aim

- To determine species diversity of a community of organisms using species richness and Simpson's diversity index
- To compare species diversity calculated using species richness and Simpson's diversity index

Optional

- To practise using formulas in spreadsheets, such as Microsoft Excel, to calculate species diversity indexes

Rationale (scientific background to the experiment)

Biodiversity, or biological diversity, refers to the variety of organisms living within a particular area. Biodiversity can be measured by the number and diversity of species, the extent to which a particular habitat or ecosystem varies, or the amount of genetic diversity within a species. The more diverse an area is, the more likely it is to withstand environmental pressures or disasters. Biodiversity is often used as an indication of the health of a habitat.

There are several ways to measure biodiversity. Two common methods are calculating species richness and calculating the Simpson's diversity index. Species richness is a simple measure of biodiversity and is the number of different species in a given habitat. The more species present in a community, the more 'rich' or biodiverse it is. Simpson's diversity index takes into account the number of different species in a community and their relative abundance of each species.

Simpson's diversity index is calculated using the formula:

$$\text{SDI} = 1 - \left(\frac{\Sigma n(n-1)}{N(N-1)}\right)$$

where:

n = number of organisms of one species

N = total number of organisms of all species

Using this formula, the Simpson's diversity index ranges from 0 to 1. A Simpson's diversity index of 0 means that there is no diversity; that is, all of the organisms in a particular area are from the one species. The closer the Simpson's diversity index is to 1, the more diverse the community is.

Timing

60 minutes

Materials

- images showing organisms in four different communities
- calculator

Optional

- Microsoft Excel or another spreadsheet program
- sampling equipment (e.g. quadrats)

Method

Risk assessment

Assessment of risks include chemical hazards and physical hazards. Before you commence this practical activity, you must conduct a risk assessment. Complete the template in your *Pearson Biology 12 Queensland Skills and Assessment* book or download it from your eBook.

1. Count the number of organisms and species in each community and record the data in the table.
2. Alternatively, you could use sampling techniques, such as quadrats, to count the number of organisms and species in habitats in or around the school.
3. Calculate species richness for each community.
4. Calculate the Simpson's diversity index for each community.
   This can be done using the tables below. Alternatively, you can practise using a spreadsheet, such as Excel, to calculate Simpson's diversity index. Tutorials are available on YouTube.

Analysing

Raw data

1. Complete the table below.

Raw data

Species	Number of individuals			
	Community 1	Community 2	Community 3	Community 4
A				
B				
C				
D				
E				
Total				

2 From the raw data, predict the order in which the communities will be listed, from the most diverse to the least diverse.

Processed data

3 Calculate the Simpson's diversity index for each community.

Community 1

Species	n	$n-1$	$n(n-1)$
A			
B			
C			
D			
E			
Total	$N =$		$\sum n(n-1)=$

$$\text{SDI} = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right) =$$

Community 2

Species	n	$n-1$	$n(n-1)$
A			
B			
C			
D			
E			
Total	$N =$		$\sum n(n-1)=$

$$\text{SDI} = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right) =$$

Community 3

Species	n	$n-1$	$n(n-1)$
A			
B			
C			
D			
E			
Total	$N =$		$\sum n(n-1)=$

$$\text{SDI} = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right) =$$

Community 4

Species	n	$n-1$	$n(n-1)$
A			
B			
C			
D			
E			
Total	$N =$		$\sum n(n-1)=$

$$\text{SDI} = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right) =$$

4 Complete the table below for the two measures of biodiversity.

Measures of biodiversity for four communities

Community	**Species richness**	**Simpson's diversity index**
1		
2		
3		
4		

➤ **Reflect and check that your data analysis demonstrates these characteristics**

- ☐ Effective investigation of phenomena is demonstrated by the collection of sufficient and relevant raw data
- ☐ Accurate application of algorithms, visual and graphical representations of data is demonstrated by appropriate processing and presentation of data to aid the analysis and interpretation of data

Analysis

5 Explain the difference between species richness and Simpson's diversity index.

6 Identify the community that had the most biodiversity.

7 Use the Simpson's diversity index to identify which community had the least biodiversity. Explain why the Simpson's diversity index was low for this community.

8 Explain whether species richness and Simpson's diversity index always correlated. In other words, did the communities with the highest species richness scores have the highest Simpson's biodiversity index? Explain why or why not.

➤ Reflect and check that your analysis demonstrates these characteristics

- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough and appropriate error analysis
- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough identification of relevant trends, patterns and relationships
- ☐ Insightful and valid interpretation of evidence is demonstrated by drawing a valid and defensible conclusion based on the analysis

Interpreting and communicating

Conclusion

1 Summarise the relationship between species richness, species abundance and the biodiversity of a particular community or habitat.

Improvements

2 If you were to repeat this experiment, identify the steps that you would do differently.

Consider how you:

a might change the methodology

b might improve your technique

c could reduce error and uncertainty.

Extension

3 Studies of biodiversity are an important component of assessing environmental health as well as the impact human activities have on environments. Explain why environmental impact studies are conducted for mining and petroleum/gas projects.

4 Use a spreadsheet, such as Microsoft Excel, to calculate the biodiversity index for the communities you have analysed using species evenness (*E*).

The formula for calculating species evenness is:

$$E = \frac{-\Sigma\left(\frac{n}{N}\ln\frac{n}{N}\right)}{\ln S}$$

where

n is the number of individuals of one species.

N is the total number of individuals across all species.

S is the total number of species (species richness).

ln is the natural log.

5 Use a sampling technique, such as quadrats, to calculate the species populations of two similar habitats at your school (e.g. two lawns). Calculate and compare the species diversity of the two habitats.

➤ Reflect and check that your evaluation demonstrates these characteristics

- ☐ Critical evaluation of processes is demonstrated by a discussion of the reliability and validity of the experimental process supported by evidence such as the quality of the data (as quantified in the error analysis)
- ☐ Critical evaluation of the conclusion is demonstrated by a discussion of the veracity of the conclusions with respect to the error analysis and limitations or sufficiency of the data
- ☐ Insightful evaluation of processes and conclusions is demonstrated by a suggestion of improvements or extensions to the experiment that are logically derived from the analysis of the evidence

Chapter review

WS 3.1.7 WS 3.1.8

03

KEY TERMS

abundance
abyssal
aquifer
basal cover
benthic region
biodiversity
brackish
canopy cover
consumer
distribution
ecosystem diversity
ectothermic
genetic diversity
ground cover
horizon
humus
hypoxic
intertidal zone
leaching
leaf cover
littoral zone
network
pelagic zone
percentage cover
percentage frequency
producer
relative species abundance
Secchi disc
Simpson's diversity index
species abundance
species diversity
species evenness
species richness
tolerance range
turbidity
undirected network
unweighted network

KEY QUESTIONS

Retrieval

1 The conditions in the deeper areas of the Great Barrier Reef are such that most corals are unable to survive there. Identify the term that describes the conditions over which a species of coral could survive.

A ideal range
B optimal range
C survival range
D tolerance range

2 Explain how a quadrat can have a percentage cover greater than 100%.

3 Describe the difference in oxygen availability in terrestrial and aquatic environments.

4 Explain what is meant by:

a genetic diversity
b biodiversity

Comprehension

5 A student performed a study of the local park and listed all of the plant species present. The student named 60 different species. Identify the measure of biodiversity that the student has calculated.

A species diversity
B species richness
C species evenness
D species abundance

6 Identify the community showing the greatest species evenness. Each community has 1000 members in total, spread between four species.

Numbers of individuals of each species in each area surveyed

	Area	Species 1	Species 2	Species 3	Species 4
A	1	100	400	200	300
B	2	248	246	253	253
C	3	115	333	498	54
D	4	333	333	333	1

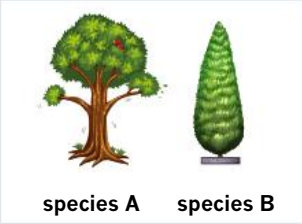
7 Explain why the intertidal zone is a challenging habitat for the organisms that live there.

8 Explain how habitat fragmentation affects biodiversity.

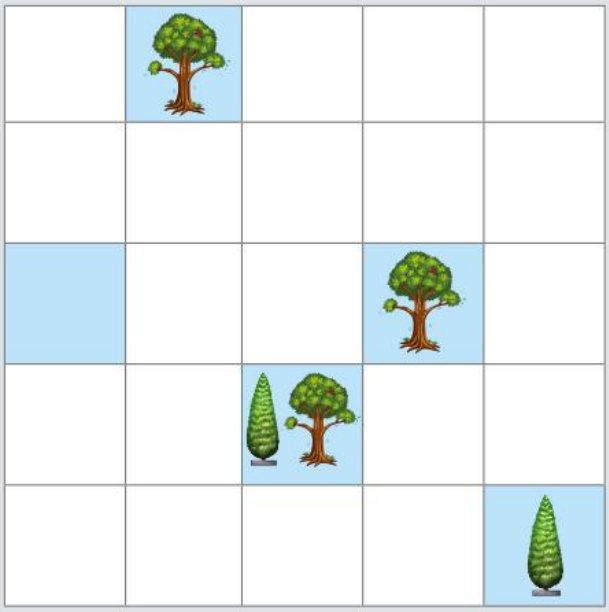
9 Many of the techniques used to assess biodiversity involve sampling. Discuss the advantages and limitations of sampling as a means of assessing ecosystems.

Analysis

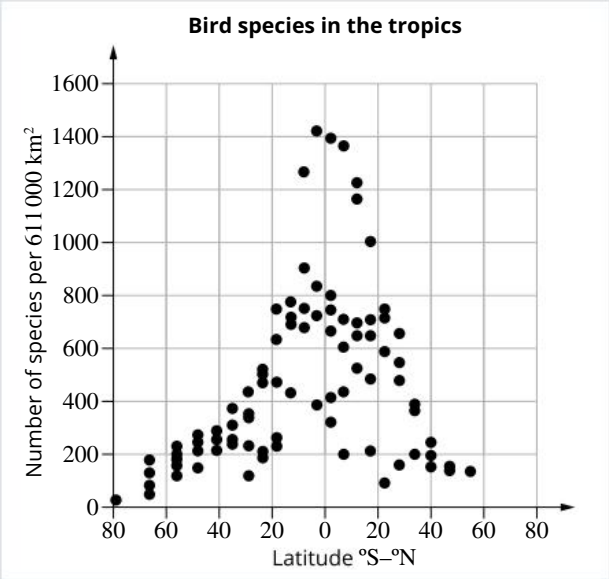
10 An area was sampled using quadrats (shaded squares) for the presence of species A and species B.



Calculate the percentage frequency for both species A and species B.

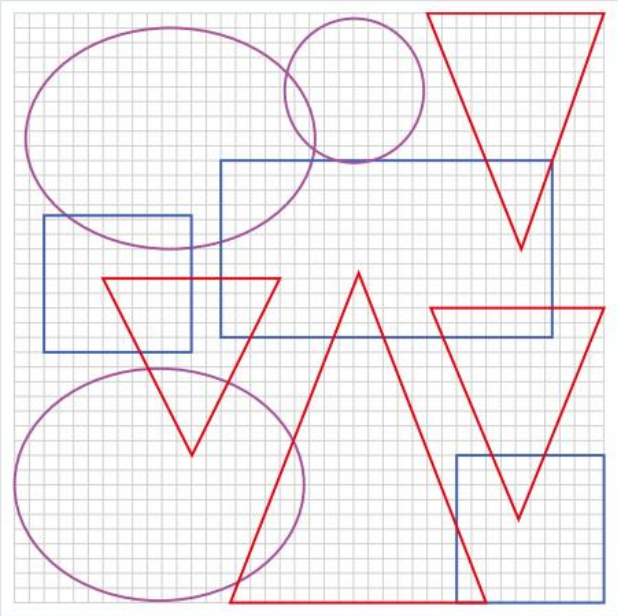


11 Draw conclusions about bird species diversity from the graph below.



12 A student surveyed a quadrat and found that it contained three species of plant: species A (blue rectangles), species B (red triangles) and species C (purple circles). The student marked the positions of the plants on a grid.

a Calculate percentage cover for each species and the total for the quadrat. The graph paper consists of 40 × 40 of the smallest squares.



b Explain a limitation in calculating percentage cover, which can result in two students using the same technique but arriving at different answers.

Use the following information to answer questions 13 and 14.

The bird numbers at Lake Yumberarra were surveyed over a number of years. The results for two of these years, 1998 and 2002, are shown in the table below.

Bird species, Lake Yumberarra, 1998 and 2002

Species	Number of individuals	
	1998	2002
blue-billed duck	10	23
freckled duck	0	124
black swan	109	4
Australian wood duck	3	0
Australasian shoveler	217	3
grey teal	531	291
pink-eared duck	17	21
Australian pelican	35	8
great egret	0	1
yellow-billed spoonbill	5	13

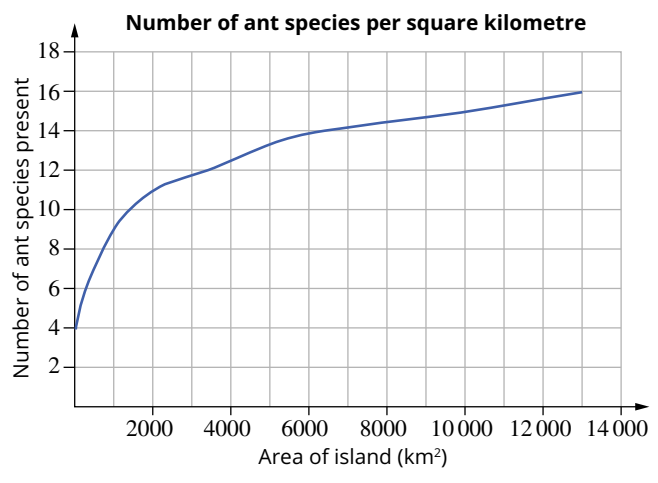
13 **a** Calculate species evenness for the waterbirds for each of the two years at Yumberarra.

b Explain which year showed more species evenness.

14 **a** Calculate species diversity for the two years at Yumberarra using Simpson's diversity index.

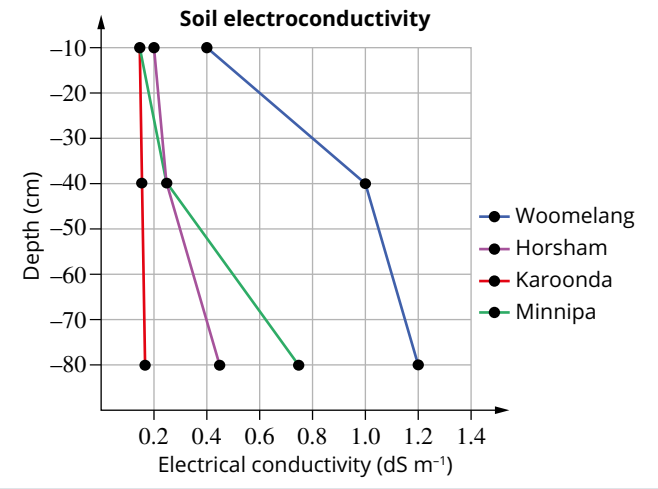
b Identify which of the communities is most diverse. Justify your conclusion.

15 The graph below shows the number of species of ant in comparison to land area size on isolated islands in the region of New Guinea. The line shown is a line of best fit. Twenty-five islands were surveyed.



Explain how these results support the hypothesis that habitat fragmentation leads to loss of biodiversity.

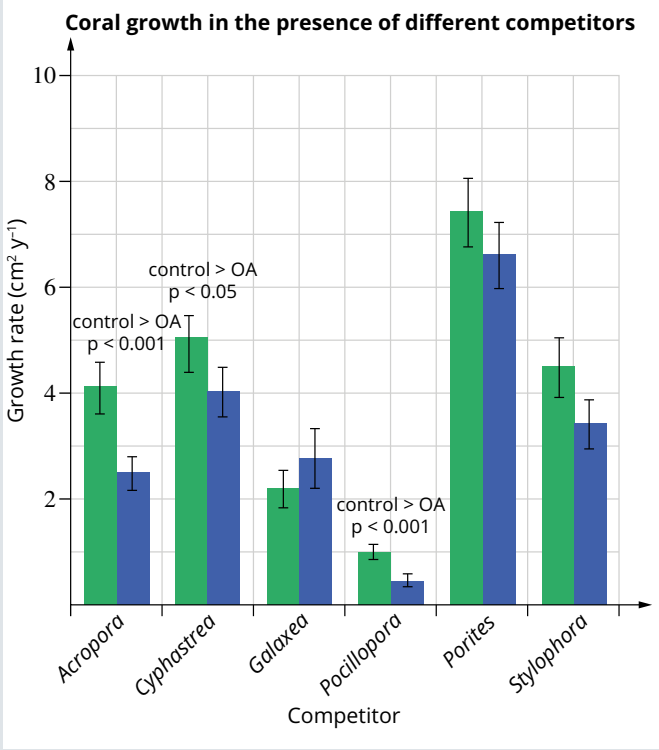
16 Soil conductivity tests were carried out at four different sites. Two were in Victoria (Woomelang and Horsham) and two were in South Australia (Karoonda and Minnipa). The results of the tests are shown on the graph below.



Discuss the implications of these results for plant biodiversity in each of these areas.

17 The graph below shows the ability of various coral species to influence the growth of their competitors. Numerous competitors were grown under controlled conditions (competitor, on the *x*-axis) and then in the presence of specific species of corals (various labels identified under the *x*-axis). *Porites* reduced the growth of its competitors the least, while *Pocillopora* decreased the growth of its competitors the most.

The effects of ocean acidification (OA, reduced pH) from atmospheric carbon dioxide absorption, which forms carbonic acid, was also tested by repeating the experiment under conditions with a lower pH. The green columns show the effect on competitors' growth rate under current ocean conditions (pH 8.1), and the blue columns are simulated ocean acidification conditions (pH 7.6). Error bars show standard error.



a Describe the effect of decreased pH on the growth of the coral.

b Explain how ocean acidification is likely to impact reef species richness and evenness.

Knowledge utilisation

18 The Great Barrier Reef has declined in coral coverage since 1985. The results of a study into that decline are shown in the table below.

Estimated rates (%y^{-1}) and standard error (in brackets) of (i) decline, growth and total mortality of coral cover and (ii) total coral mortality by crown-of-thorns sea stars (COTSS), cyclones and bleaching

		GBR	North	Centre	South
i	decline	0.53 (0.08)	0.11 (0.14)	0.44 (0.08)	1.04 (0.16)
	growth	2.85 (0.26)	2.07 (0.44)	2.78 (0.26)	2.34 (0.52)
	total mortality	3.38 (0.19)	2.18 (0.35)	3.22 (0.18)	3.38 (0.44)
ii	COTSS mortality	1.42 (0.17)	0.77 (0.25)	1.54 (0.24)	1.59 (0.27)
	cyclone mortality	1.62 (0.22)	1.05 (0.23)	1.29 (0.14)	1.75 (0.32)
	bleaching mortality	0.34 (0.08)	0.36 (0.13)	0.39 (0.09)	0.04 (0.11)

a Explain why the decline is less than the mortality.

b Consider the causes of reef loss and propose strategies to conserve the Great Barrier Reef.

19 The interaction between sharks and dugongs is just one set of interactions occurring at Shark Bay, Western Australia. Investigate the research into the Shark Bay ecosystem and write a report that could appear in the nature section of a newspaper or magazine.

20 Research the findings of the Biological Dynamics of Forest Fragments Project. Present the results of your research as a poster.

CHAPTER 04

Ecosystem dynamics

Ecosystems are dynamic; their biotic and abiotic components are constantly changing. The changes within an ecosystem are not inherently negative, in fact, the ability of organisms to respond to external factors ensures the longevity of an ecosystem. However, some changes, such as deforestation caused by humans, are so sudden and dramatic that organisms living within an ecosystem are unable to respond, and the ecosystem itself becomes unstable.

By the end of this chapter, you will understand the different types of relationships between species in an ecosystem and the ways in which species are interdependent. You will also learn about the factors that influence a population's size, density and distribution within an ecosystem. You should be able to identify the role of keystone species and identify some of the factors that make them crucial to ecosystem stability. You will consider the factors that determine both the rate of growth of a population and what causes growth rates to level off. You should also understand why populations can suddenly undergo rapid growth or rapid decline. You will study the ways that materials such as carbon and nitrogen, as well as energy, are cycled through ecosystems. You will learn how to calculate energy transfers and estimate population size. Finally, you will consider the processes leading to change of ecosystems over time and the impact that human activities can have on the long-term viability of the various ecosystems of the Earth.

Syllabus subject matter

WS 3.2.1

Topic 2 • Ecosystem dynamics

FUNCTIONING ECOSYSTEMS

- sequence and explain the transfer and transformation of solar energy into biomass as it flows through biotic components of an ecosystem including:
  - converting light to chemical energy
  - producing biomass and interacting with components of the carbon cycle
- analyse and calculate energy transfer (food chains, webs and pyramids) and transformations within ecosystems, including:
  - loss of energy through radiation, reflection and absorption
  - efficiencies of energy transfer from one trophic level to another
  - biomass
- construct and analyse simple energy-flow diagrams illustrating the movement of energy through ecosystems, including the productivity (gross and net) of the various trophic levels
- describe the transfer and transformation of matter as it cycles through ecosystems (water, carbon and nitrogen)
- define ecological niche in terms of habitat, feeding relationships and interactions with other species
- understand the competitive exclusion principle

- analyse data to identify species (including microorganisms) or populations occupying an ecological niche
- define keystone species and understand the critical role they play in maintaining the structure of a community
- analyse data (from an Australian ecosystem) to identify a keystone species and predict the outcomes of removing the species from an ecosystem.

POPULATION ECOLOGY

- define the term carrying capacity
- explain why the carrying capacity of a population is determined by limiting factors (biotic and abiotic)
- calculate population growth rate and change (using birth, death, immigration and emigration data)
- use the Lincoln index to estimate population size from secondary or primary data
- analyse population growth data to determine the mode (exponential growth J-curve, logistic growth S-curve) of population growth
- discuss the effect of changes within population-limiting factors on the carrying capacity of the ecosystem.

CHANGING ECOSYSTEMS

- explain the concept of ecological succession (refer to pioneer and climax communities and seres)
- differentiate between the two main modes of succession: primary and secondary
- identify the features of pioneer species (ability to fixate nitrogen, tolerance to extreme conditions, rapid germination of seeds, ability to photosynthesise) that make them effective colonisers
- analyse data from the fossil record to observe past ecosystems and changes in biotic and abiotic components
- analyse ecological data to predict temporal and spatial successional changes
- predict the impact of human activity on the reduction of biodiversity and on the magnitude, duration and speed of ecosystem change.

SCIENCE AS A HUMAN ENDEAVOUR

Aboriginal and Torres Strait Islander people's knowledge of ecosystem interactions and change.

MANDATORY PRACTICAL 3

Select and appraise an ecological surveying technique to analyse species diversity between two spatially variant ecosystems of the same classification (e.g. a disturbed and undisturbed dry sclerophyll forest).

Revision

Some of this revision material will be familiar to you from your junior studies. Other material is foundational information required to understand concepts outlined in the Biology General Senior Syllabus. This material does not form part of the Biology General Senior Syllabus and should not be factored as part of the notional hours for Chapter 4.

ORGANISING THE ENVIRONMENT

Organisms do not live in isolation. They interact with each other and with their physical surroundings. To understand and explain the interactions between the various organisms and their environment, ecologists study each species, how they relate to each other, other species and their abiotic environment in a hierarchy of organisation. Organisms interact with one another in many ways. Some eat others, some others compete for resources, such as food, space or mates, and some live in mutually beneficial relationships with other organisms.

Environments can be studied at different levels. These biological levels of organisation follow a highly structured hierarchy of living things:

- individual (one member of one species)
- population (a group of individuals of the same species living in one area at one time)
- community (a group of interacting species)
- ecosystem (a group of interacting species and their abiotic environment)
- biome (all the ecosystems having common characteristics and environments with shared physical climates)
- biosphere (all the regions of Earth inhabited by living things).

Communities

Organisms of a particular species live together in populations. Different kinds of **populations** (for example, lyrebirds, tree ferns and insects) live together in communities. A **community** is an ecological grouping of different species that live together in a particular place at a particular time and interact with one another. For example, the insects, spiders, mosses, fungi and bacteria on a dead tree trunk make up a community of organisms that live and interact with one another. Spiders feed on insects, insects feed on one another as well as the fungi, and mosses catch and hold the rainwater that all of the organisms require and provide shelter for the spiders and insects. The fungi and bacteria recycle the nutrients in the community by breaking down dead spiders, insects and mosses.

Ecosystems

An ecosystem is a system formed by a community of living organisms interacting with one another in a particular place at a particular time, and also interacting with their physical surroundings. To be defined as an ecosystem, a system must be self-sustaining. This means that it can be maintained into the future largely without inputs from outside the system. For this reason, artificial environments, like aquariums, are not considered to be ecosystems as they rely on inputs from outside and external interventions to remove wastes.

Ecosystems can be almost any size. An ecosystem can be as small as a dead tree trunk, or it can be large like the Daintree rainforest. The Daintree covers approximately 1200 km^2 (Figure 4.0.1).

Ecosystems also vary in complexity. Tropical forests like the Daintree are the most complex land ecosystem and contain the greatest number of species. A desert ecosystem is one of the simplest. Cities and towns are urban ecosystems.

FIGURE 4.0.1 The Daintree rainforest is part of a World Heritage Area. It is an ecosystem that includes a large number of species.

Biomes

A **biome** is a group of communities of living things that have a particular set of adaptations to a specific environment, along with the environment that they inhabit. Biomes can be very widespread. They have similar climates and geographical features but may be distant from each other, such as tropical rainforest (Figure 4.0.2). Biomes can be terrestrial and aquatic.

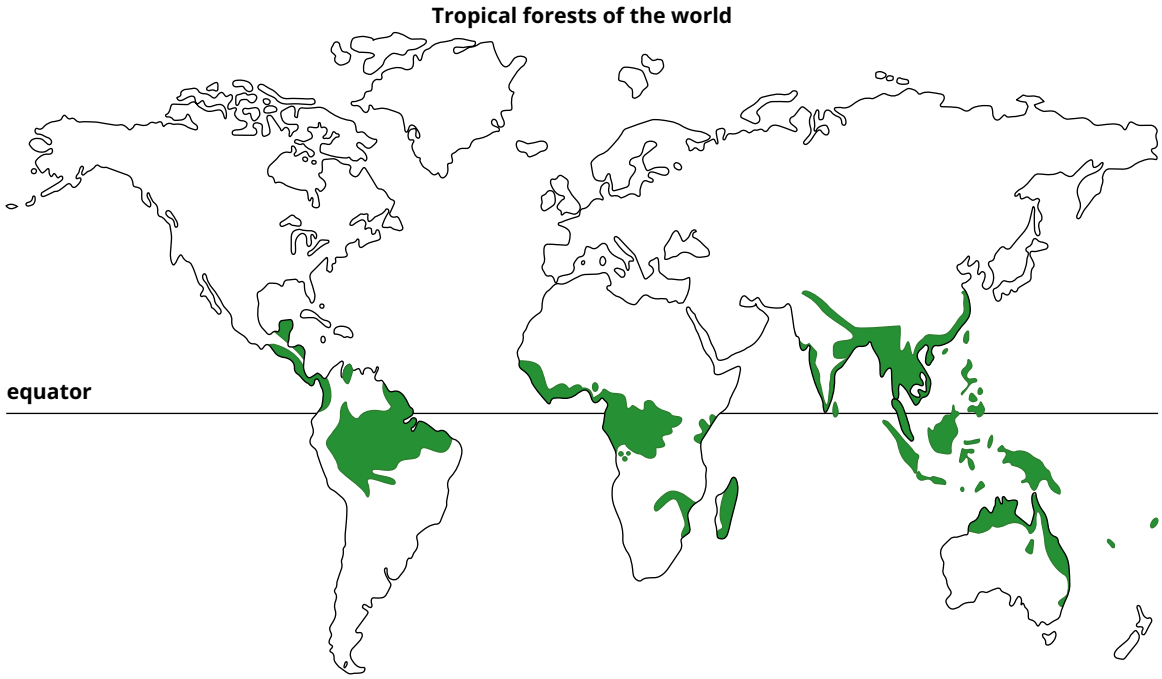


FIGURE 4.0.2 The tropical rainforest biome (shown in green) is found close to the equator. It is characterised by warm temperatures and high rainfall.

Terrestrial biomes

Terrestrial biomes include:

- tundra (areas of permanently frozen ground, especially near the poles)
- tropical rainforest
- savanna (tropical and subtropical grasslands)
- taiga (conifer forests that are swampy and found in areas below the Arctic)
- temperate forest
- temperate grassland
- alpine (high mountain areas)
- chaparral (area of shrubs, small trees and thorny thickets usually with dry summers and wet winters)
- desert.

Aquatic biomes

Aquatic biomes include:

- freshwater wetlands (lakes and rivers)
- open ocean
- coral reefs
- estuaries (tidal areas where large rivers empty into the ocean).

The biosphere

The largest and most complex ecosystem of all is the **biosphere**, which is the sum of all ecosystems on Earth. The biosphere includes all those parts of Earth that are accessible to living organisms, including oceans, rivers, lakes (**hydrosphere**), soil and rocks (**lithosphere**) and air (**atmosphere**). The biosphere occupies a thin layer of atmosphere, hydrosphere, and a thin layer of lithosphere (Figure 4.0.3).

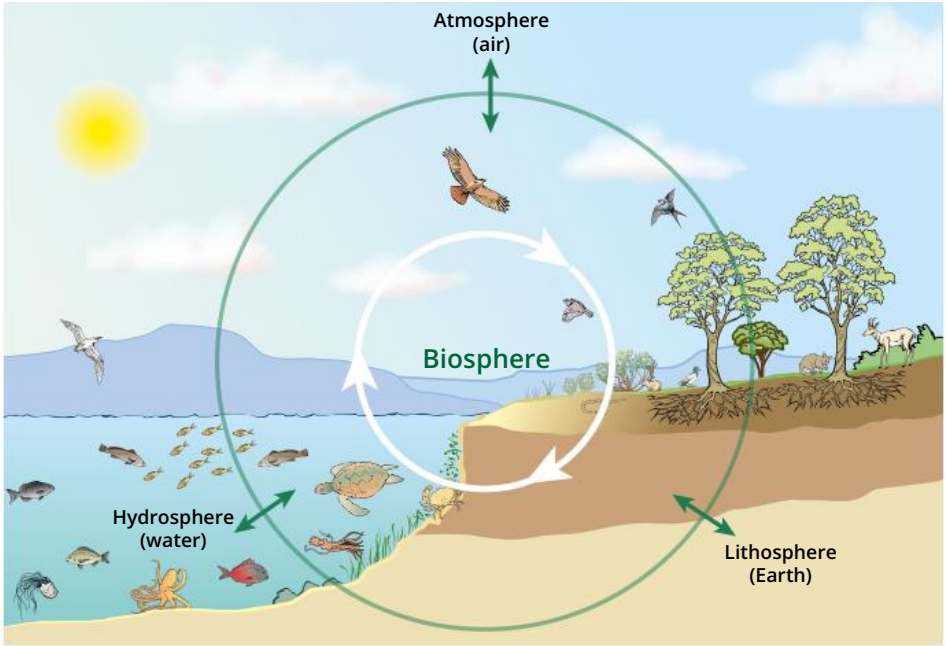


FIGURE 4.0.3 The biosphere is the portion of the Earth inhabited by living things. It occupies parts of the atmosphere, hydrosphere and lithosphere.

An example of how these levels of organisation apply to a particular organism, the clownfish, is shown in Figure 4.0.4.

Individual: Single organism such as a plant, animal or fungus, or unicellular organism. The clownfish can be studied on its own.

Population: A group of organisms of the same species, living together in a defined geographical area. Clownfish live in groups in the tentacles of a sea anemone. The group of clownfish is a population.

Community: The clownfish lives in close and mutually beneficial relationship with the sea anemone. The clownfish, sea anemone and all the other living things of the coral reef form a community.

Ecosystem: The coral reef is an ecosystem. It contains a community of organisms, such as clownfish and anemones, which interact with each other and their abiotic environment. Within the ecosystem, each organism has its own habitat that is both the living and non-living elements of its environment.

Biome: The coral reef biomes inhabited by the clownfish primarily exist between the tropics. They are generally in warm, shallow marine environments.

Biosphere: The biosphere covers all the regions of Earth that are inhabited by living things. The Earth could be considered to be one huge ecosystem as all of the various living and non-living parts of the Earth ultimately affect each other.

FIGURE 4.0.4 The hierarchy of organisation of living things from the simplest (the individual) to the most complex (the biosphere).

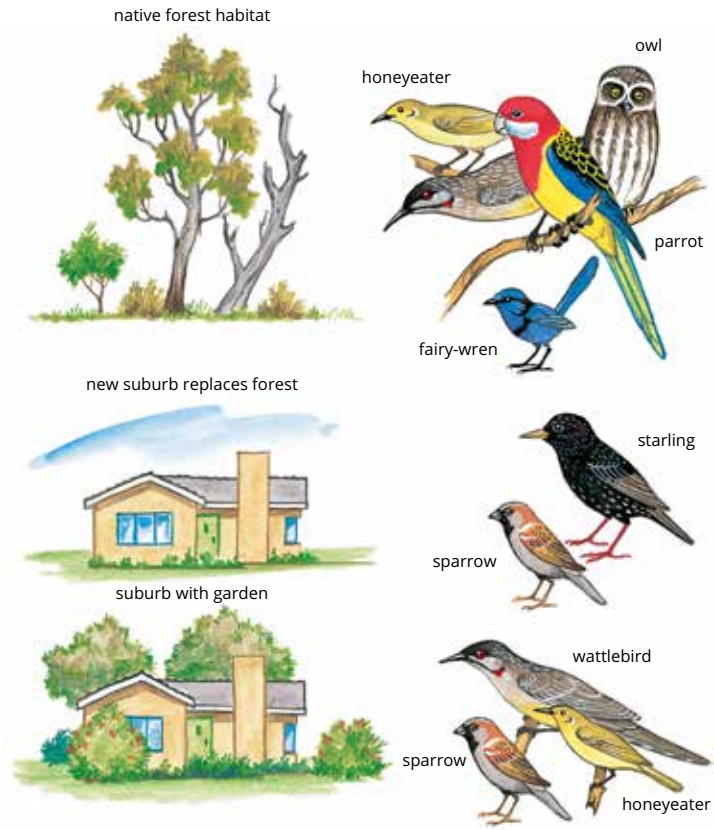


FIGURE 4.0.5 The bird community in a new suburb consists of sparrows and few native species. Gardens that include native plants provide a habitat for a greater variety of birds.

Habitat

The habitat of rock orchids, as the name suggests, is rock outcrops in forests. The habitat of a fragrant water lily is still, shallow lakes and ponds with silty bottoms. The bush rat lives in small burrows hidden under fallen logs or rocks in dense vegetation on the floor of forests, while the swamp rat lives mainly in grasslands and heathland close to water. Different habitats support different species (Figure 4.0.5).

Each ecosystem contains many habitats. For example, the Daintree ecosystem contains rivers, lakes, dense forests and open areas, each of which provides a different habitat.

Some organisms live in only one type of habitat found in a small number of locations For example, hillsides and creek banks with soils formed from the weathering of basaltic rocks on Queensland's Sunshine Coast are the only known habitat of the Buderim holly. The Sydney rock oyster, although found at many locations along the eastern coast of Australia, is always attached to rocks in sheltered estuaries and bays in the intertidal zone (Figure 4.0.6).

Other organisms live in habitats found in a greater range of locations. Flying foxes are fruit-eating bats that inhabit forests, paperbark swamps and mangroves from Queensland to Victoria (Figure 4.0.7). They can be found roosting in the middle and upper canopy of trees in most areas where trees that provide the flowers and fruits that form their food source are also found.

Some organisms move from one habitat to another according to seasonal changes. For example, pelicans prefer to live where large areas of open water are fringed by land with sparse vegetation. These conditions are met in many coastal regions and inland during times of rain and flood, and pelicans move to take advantage of both.

FIGURE 4.0.6 Sydney rock oyster.

FIGURE 4.0.7 Flying foxes inhabit forests in eastern Australia.

Microhabitat

Within a habitat there are smaller areas known as microhabitats. A microhabitat could be a burrow, a tree canopy or even inside other organisms.

In a microhabitat, an organism experiences a slightly different environment compared to the overall habitat, such as a lower temperature, more moisture, less sunlight or more humidity. This variation in environmental conditions can be essential for the organism to survive.

The moist trunk of the tree fern in a wet forest is the microhabitat of many mosses, liverworts, filmy ferns, fungi, spiders and insects. The trunk of the tree fern is shaded by its umbrella of large fern leaves, is soft and able to absorb and hold much water (Figure 4.0.8). The tree fern's trunk is a different microhabitat from the trunk of trees such as silver wattles in the same forest habitat.

FIGURE 4.0.8 Tree fern.

4.1 Energy transfer

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand that all organisms need a carbon source in order to live, grow and reproduce
- understand how different organisms obtain the organic molecules that they need for survival
- understand how energy is transferred and lost between trophic levels in ecosystems
- understand the ways in which organisms interact with each other
- understand how biotic (living) and abiotic (non-living) factors such as light, temperature and inorganic molecules influence ecosystem productivity
- understand how water, carbon and nitrogen are cycled on Earth.

This module considers the processes by which energy and matter pass through ecosystems and how organisms obtain and use their matter and energy requirements. In this module, you will also examine the factors, both living and non-living, that influence the capture and release of energy by organisms in ecosystems.

OBTAINING NUTRIENTS AND ENERGY

Although there is an enormous diversity of living organisms in the world, living organisms have many processes and requirements in common. Whether organisms are unicellular or multicellular, or live at the bottom of the ocean or in a rainforest, they all need to take in nutrients and water, exchange gases, obtain energy and remove waste products. And ultimately, most biological systems and organisms rely primarily on one source of energy for their survival: light, generally sunlight (Figure 4.1.1).

Organisms can be divided into two groups depending on the strategies they use to obtain organic compounds.

- **Autotrophs** obtain organic compounds by converting inorganic matter. Because they produce all the organic compounds in ecosystems, they are also called producers.
- **Heterotrophs** obtain organic compounds from autotrophs or other heterotrophs. Because they consume organic compounds, they are also called consumers.

The energy stored in the bonds between the atoms in complex organic molecules, such as glucose, is not immediately accessible to the cells of organisms so it must be converted to a usable form. This usable form is the chemical **adenosine triphosphate (ATP)**. The energy in glucose is transferred to ATP during the process of cellular respiration. The energy in ATP is then used by cells for the processes of life (for example, movement, protein synthesis, immune response). Figure 4.1.2 (page 134) illustrates the interconnectedness of the processes of photosynthesis and cellular respiration and demonstrates how, ultimately, the sun is the source of nearly all energy used by living things on Earth.

FIGURE 4.1.1 Sunlight is the primary source of energy for biological systems and the organisms within them.

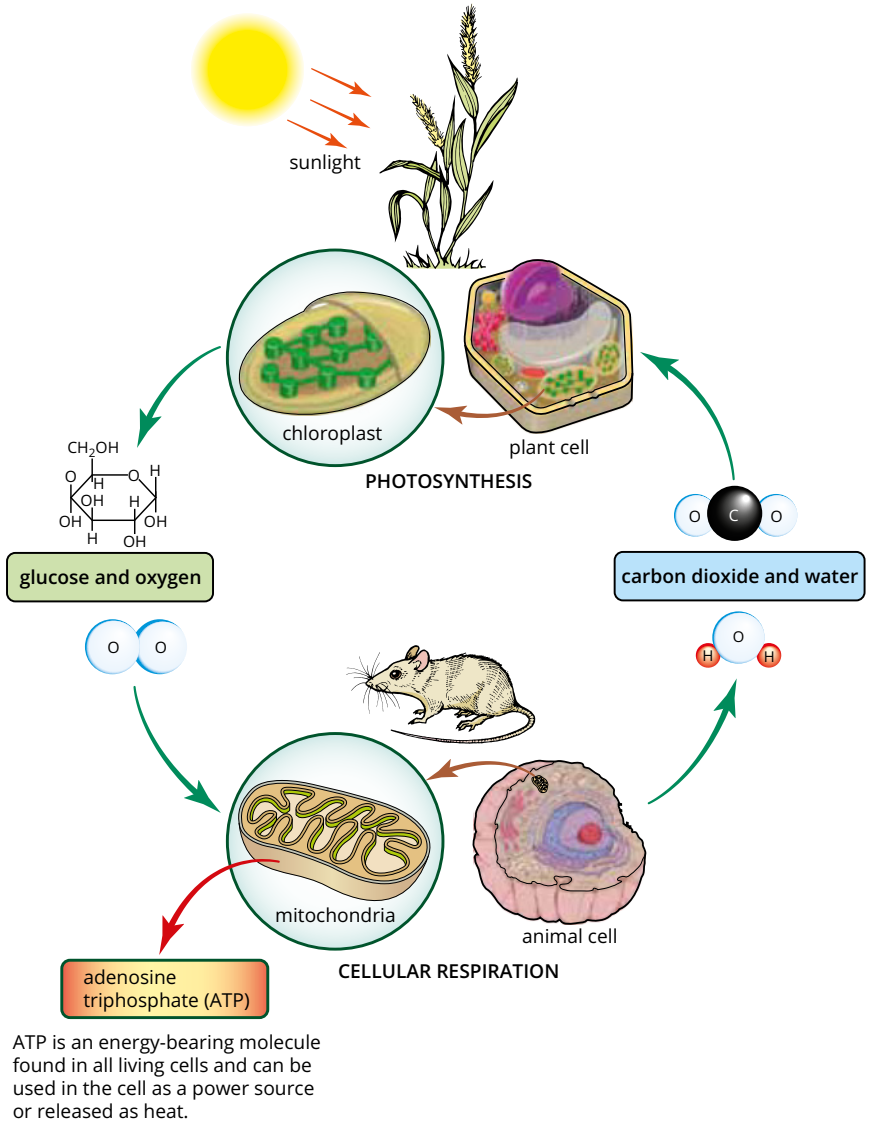


FIGURE 4.1.2 Photosynthesis and cellular respiration are central processes within biological systems. Plants convert sunlight into the organic compound glucose through the process of photosynthesis.

Autotrophs

Autotrophs (self-feeders) are able to make their own organic compounds. They use energy and inorganic molecules, such as carbon dioxide and water, which come from their physical environment, to synthesise (make) organic compounds. This process is called **carbon fixation** because the autotroph 'fixes' the inorganic carbon into organic molecules.

Autotrophs can be further divided into two groups according to how they obtain the energy required for carbon fixation. These two groups are:

- chemosynthetic autotrophs
- photosynthetic autotrophs.

The organic compounds synthesised by autotrophs, such as the carbon-based molecule glucose, are the primary source of energy for all organisms and ecosystems. They are responsible for the production of the only source of carbon-fixed organic molecules that fuel all living processes in all organisms in all ecosystems. This is why they are known as primary producers. Autotrophs are the foundation of biodiversity and abundance in ecosystems, as explained in Chapter 3.

Organic compounds contain both carbon and hydrogen. The main types are carbohydrates, proteins, nucleic acids and lipids. Inorganic compounds may contain either carbon or hydrogen, or be made up of other elements. Examples of inorganic substances include water, oxygen, carbon dioxide and nitrogen.

Chemosynthetic autotrophs

While most autotrophic species use light as their energy source, this is not always the case. **Chemosynthetic autotrophs** (**chemoautotrophs**) obtain the energy they need for carbon fixation from inorganic chemical reactions in a process known as **chemosynthesis**. All chemosynthetic organisms are prokaryotes.

One group of chemoautotrophs are the producers of the ecosystems that are found deep in the ocean, around the mid-ocean ridges. In these areas of the sea floor, the chemicals released from the Earth's crust by volcanic hydrothermal vents, especially hydrogen sulfide, are oxidised to release energy that is used to build complex organic compounds. Over 300 unique species of animals, including a large number of tube worms, have been identified in these ecosystems (Figure 4.1.3).

Oxidation is a chemical process. In the case of chemosynthetic bacteria, oxidation of hydrogen gas, hydrogen sulfide or methane occurs and energy is released for use by the bacteria in the process.

FIGURE 4.1.3 Giant tube worms growing in a hydrothermal vent deep in the Pacific Ocean. They obtain their nutrients by feeding on chemosynthetic bacteria.

Photosynthetic autotrophs

Photosynthetic autotrophs (**photoautotrophs**) are organisms that obtain the energy required for carbon fixation from light or solar energy (sunlight). They combine carbon dioxide and water using light energy to produce organic compounds in a process known as photosynthesis. In plants, the organic compound produced by photosynthesis is glucose.

Most autotrophs are photosynthetic. The typical photosynthetic organisms you might think of are green plants, but there are also photosynthetic protists, such as algae, and cyanobacteria (Figure 4.1.4).

FIGURE 4.1.4 Photoautotrophs use light energy to synthesise organic molecules from carbon dioxide and water. (a) On land, plants are the main photoautotrophs. In aquatic environments the main photoautotrophs are (b) algae such as this kelp and (c) prokaryotes called cyanobacteria.

The process of photosynthesis

Plants produce glucose by photosynthesis. **Photosynthesis** is the process in which plants and other photoautotrophic organisms obtain energy from sunlight to make their own organic compounds (Figure 4.1.5). This section will focus on photosynthesis in plants.

Simple experiments show that when plants have light, water and carbon dioxide, they make glucose in tissues that contain the green pigment, chlorophyll, such as leaves. Not all light-capturing tissues appear green, as there are other coloured pigments that are able to capture light, but they must pass the energy to chlorophyll before it can be used. The chlorophyll-containing tissues trap the energy of sunlight and convert it into chemical energy, which they store in the bonds of glucose molecules ($C_6H_{12}O_6$). This enzyme-controlled process is called photosynthesis (*photo* meaning 'light', *synthesis* meaning 'putting together'). All photosynthetic organisms, from single-celled algae to the largest trees, produce glucose in the same way.

FIGURE 4.1.5 Photosynthesis in (a) plants, (b) algae and (c) cyanobacteria provide the matter and energy for most life on Earth.

FIGURE 4.1.6 Leaves, and some stems, are green because the mesophyll cells contain many chloroplasts, the organelles in which photosynthesis takes place. Fruits (such as these blueberries) and seeds contain no chlorophyll and do not take part in photosynthesis.

Photosynthesis is called carbon fixation because carbon atoms from the air are incorporated ('fixed') into organic molecules. The process of photosynthesis is a multi-step pathway that involves a series of enzymes and chemical reactions. It is summarised by the equation:

$$6CO_2 + 12H_2O \xrightarrow[\text{energy as light}]{\text{chlorophyll}} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Only parts of the plant that contain chloroplasts, such as leaves and some stems, can undergo photosynthesis. Many parts of the plant, fruits, tree trunks and roots do not photosynthesise (Figure 4.1.6).

Efficiency of solar energy utilisation

Plants absorb light, using it as an energy source to drive the process of photosynthesis (refer to Chapter 3 of *Pearson Biology 11 Queensland* for more information about photosynthesis); however, not all species are equally efficient at light utilisation. The amount of solar energy captured by a plant varies from less than 1% to more than 6%. The efficiency of utilisation depends on the photosynthetic pathway used by the plant and the arrangement and distribution of the leaves. There are a number of different photosynthetic pathways. The two most common pathways produce either a compound containing three carbon atoms (C_3 pathway) or a compound containing four carbon compounds (C_4 pathway) as one of the first compounds formed in the production of glucose.

Of the two pathways, the C_4 pathway more efficiently uses the light that falls upon the leaves. As can be seen in Figure 4.1.7, most of the solar energy that falls on plants is not used by them. Also, the small percentage of energy that is used by the plants is mostly expended in performing the activities of metabolism. Only a small amount of energy ends up being incorporated into the structure of the plant to become part of the plant's biomass. Also, only a small amount of the light falling on a plant is captured for photosynthesis. Chloroplasts absorb light using pigments, such as chlorophyll (refer to Chapter 3 of *Pearson Biology 11 Queensland* for more information about chlorophyll). Some plants absorb a broader range of wavelengths of solar energy because they contain extra pigments. Each of these pigments is only able to absorb light in a narrow range of wavelengths, so even then only a small fraction of the available energy is absorbed.

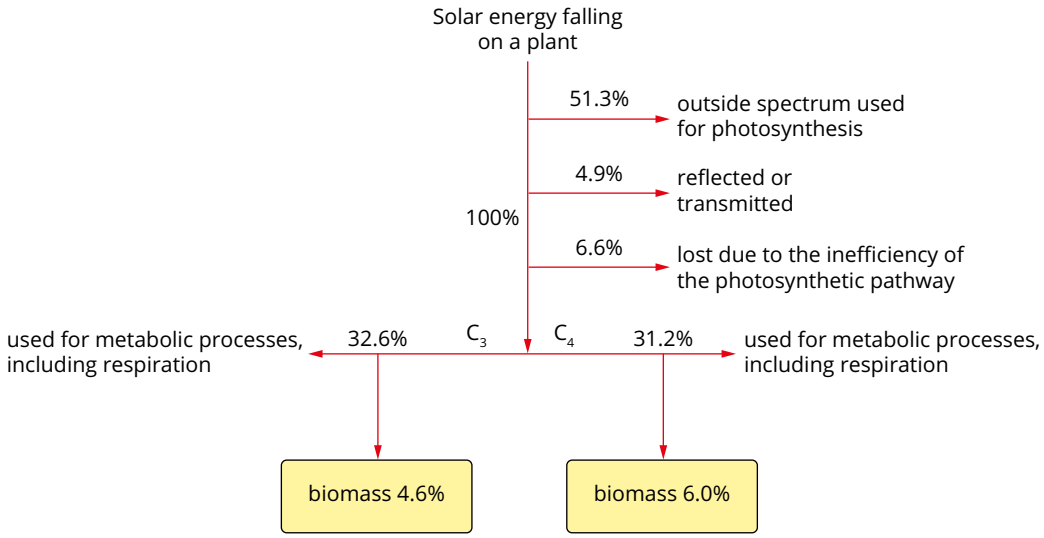


FIGURE 4.1.7 The utilisation of the solar energy falling on a plant.

Biomass is the total quantity of biological matter of a group of organisms in a given area. Biomass consists of the carbohydrates, lipids, proteins and other organic molecules that comprise the group of organisms. The energy incorporated into these organic molecules is available to other organisms that consume plants or other living things as their energy source.

Measuring biomass and productivity

The biomass of plants is measured as the amount of dry weight that accumulates in a unit area. For example, the biomass of a grass pasture is the dry weight of the grass on 1 m^2 of land. In other words, if you harvest, dry and weigh all the plants in 1 m^2, you will have a measure of biomass. Biomass can refer to the mass of living matter per unit area (g m^{-2}) or the equivalent amount of chemical energy bound in the mass of living matter (kJ m^{-2}). Dry weight is measured because water in tissue contains no usable energy. From this description, you can see why plant biomass is also called the 'standing crop'—the amount of plant matter standing at any one time.

Biomass production can also be measured over time. It is expressed as the amount of biomass per unit area per unit time (for example, g m^{-2} y^{-1}) or the amount of chemical energy bound in the biomass per unit time (for example, kJ m^{-2} y^{-1}). **Gross primary productivity** is the rate at which solar energy is utilised to fix carbon into organic molecules. On Earth, the average gross plant production is 2.7×10^4 kJ m^{-2} y^{-1}.

Net primary productivity is the rate at which biomass accumulates. It is calculated by subtracting the energy used in metabolism from the gross primary production:

net primary production = gross primary production – metabolism.

The average net primary production of the Earth is about 2.07×10^4 kJ m^{-2} y^{-1}, but the productivity of different biomes (types of ecosystem) can vary considerably from the average. Figure 4.1.8 shows the average biomass productivity of the various biomes.

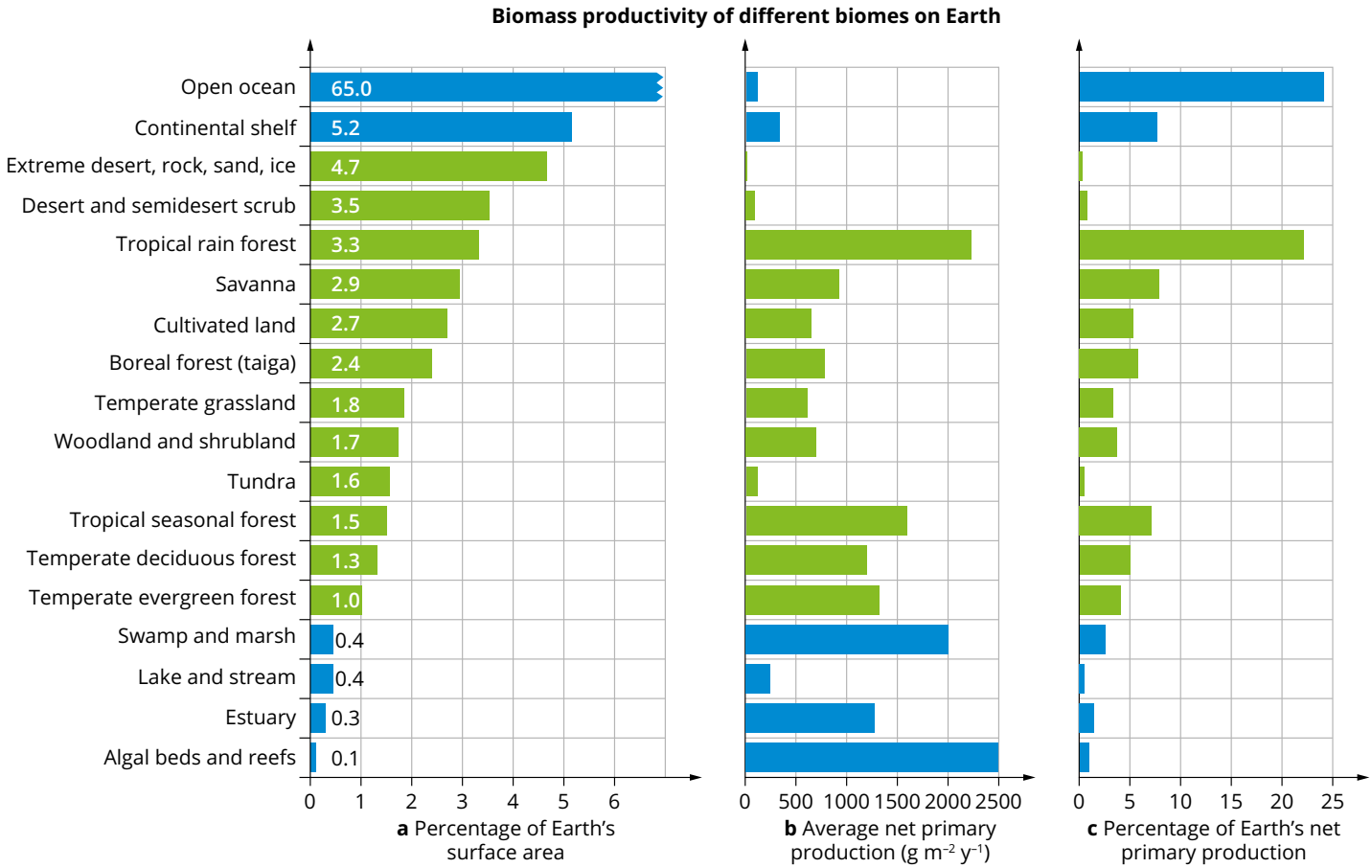


FIGURE 4.1.8 Biomass productivity of different biomes on Earth. Blue bars represent aquatic biomes and green bars represent terrestrial biomes. Note that the open ocean is responsible for a large percentage of the Earth's production but is quite low in productivity per square metre. Its large productivity is a consequence of its huge area; around 65% of the Earth's surface.

Because biomass productivity of plants primarily depends on photosynthesis, vegetation density and light availability significantly impact rates of productivity. However, other factors such as temperature and rainfall also affect productivity rates. Consequently, **primary productivity** varies with the change of seasons. The difference is particularly significant in the temperate zones, where seasonal differences in rainfall and temperature are large. The constant temperatures and high rainfall of the tropics means that these areas have high productivity all year.

As can be seen in Figure 4.1.9, primary productivity varies due to seasonal and yearly factors. Even at the same time of the year, in different years, productivity varies because contributing factors, such as rainfall, are not always the same.

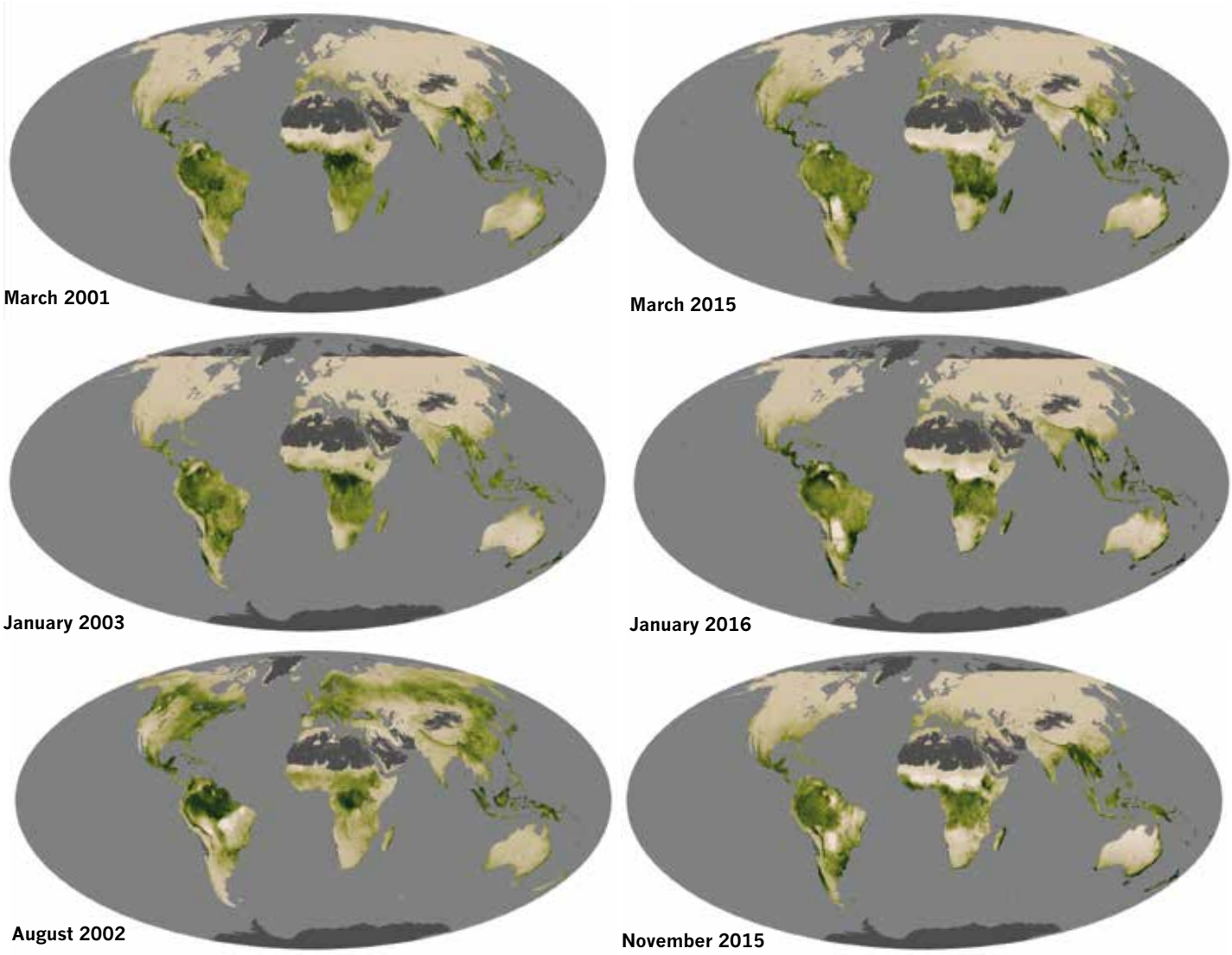


FIGURE 4.1.9 The maps show net primary productivity of the Earth. The images were generated using data from a satellite and show carbon dioxide utilisation. The carbon usage of the Earth varies from 0 g m^{-2} d^{-1} (lightest tan colour) to 6.5 g m^{-2} d^{-1} (darkest green colour).

Heterotrophs

Heterotrophs ('other feeders') are also called consumers because they are unable to make their own food. Unlike autotrophs, heterotrophs cannot use simple inorganic substances to make organic compounds. Instead, they obtain the organic compounds they need by consuming other organisms or their products. Figure 4.1.10 shows the flow of energy from autotrophs to heterotrophs in an ecosystem.

All heterotrophs depend directly or indirectly on autotrophs for nutrients and energy. All animals and fungi are heterotrophs. Some bacteria and many protists (protozoa) are also heterotrophs. Because most heterotrophs feed on particular organisms, they can be further subdivided into groups based on their diet.

Heterotrophs obtain organic compounds by consuming other organisms.

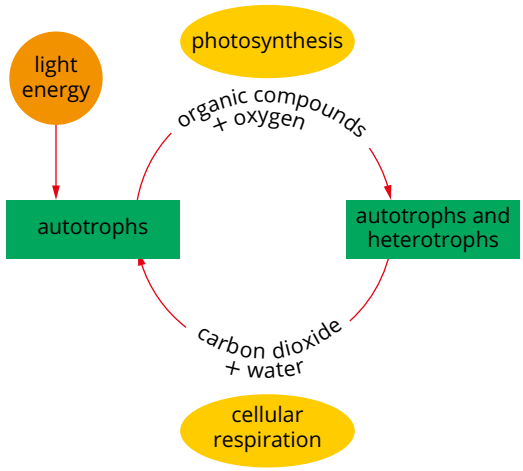


FIGURE 4.1.10 Autotrophic organisms make the organic compounds they require by combining simple inorganic compounds from their environment. Heterotrophic organisms obtain the organic compounds they require by consuming other organisms or the wastes of other organisms.

FIGURE 4.1.11 The purple swamphen (*Porphyrio porphyrio*) eats both the seeds and soft stems of rushes, and insects that also feed on the rushes.

FIGURE 4.1.12 The eastern blue-tongued skink (*Tiliqua scincoides scincoides*) is omnivorous, consuming a range of plants and animals, including snails and beetles.

There are five main categories of heterotrophs:

- **herbivores** (eat producers)
- **carnivores** (eat herbivores or other carnivores)
- **parasites** (obtain nutrients directly from other living things, called **hosts**, generally without killing them directly)
- **detritivores** (eat small particles of dead organic matter that has collected as **detritus** or organic waste, such as faeces)
- **decomposers** (secrete digestive enzymes that digest surrounding organic material, which they then absorb).

If a carnivore hunts and kills its food, it is also called a **predator**. If a carnivore eats animals that are already dead, it is a **scavenger**. Many carnivores are both predators and scavengers.

Detritivores and decomposers compete for the same food sources but digest them using different processes. Detritivores have internal digestion followed by absorption of the nutrients, which is usual in animals. Decomposers use external digestion followed by absorption of the simple nutrients, such as amino acids, that result from the digestion.

Some organisms use a mixture of strategies for obtaining nutrients. One such group is **omnivores**. Omnivores eat both plants and animals. For example, a swamphen eating a soft rush stem is using an autotroph directly as a food source. That swamphen might also eat an insect that feeds on the rush, so the swamphen also depends indirectly on the plant for its food (Figure 4.1.11).

Omnivores do not tend to specialise in a food source. They are opportunistic eaters, eating foods that are easily available to them. Humans, bears, bilbies, emus and lizards, such as the blue-tongued skink (Figure 4.1.12), are all omnivorous.

Most carnivores capture and kill other animals so they are predators, but many also act as scavengers.

TRANSFERRING ENERGY THROUGH ECOSYSTEMS

In terrestrial ecosystems, plants and other photosynthetic organisms capture light energy and incorporate the energy into complex organic molecules. All other organisms rely on these complex molecules for their energy needs. Transfers of energy and biomass occur between trophic levels. These transfers are illustrated using food chains and food webs. Pyramids of biomass and energy can also be used to illustrate the amounts of organic material and energy at each trophic level.

Terms such as primary, secondary, tertiary and quaternary are also used to describe organisms in food chains. These are terms that mean first, second, third and fourth. For example, a secondary consumer is the second consumer in a food chain, while a secondary carnivore is the second carnivore in the food chain. Some terms are interchangeable for the roles organisms play in food chains and food webs. Throughout this module, you will see different representations of food chains and food webs, and terms used interchangeably.

Table 4.1.1 provides a guide to the roles and positions of organisms in ecosystem interactions. **Trophic levels** refer to energy transfer and consumer levels refer to organism interactions. An individual species may occupy a position in more than one trophic or consumer level. This will depend on how it obtains its energy and what organisms it is consuming. More detail about trophic levels will be provided throughout this module.

TABLE 4.1.1 Trophic level and organism interaction guide

Fifth trophic level	Quaternary consumers/fourth-order consumers (omnivores and carnivores) • consume tertiary consumers and may also consume secondary consumers and/or primary consumers, e.g. foxes	Heterotrophs
Fourth trophic level	Tertiary consumers/third-order consumers (omnivores and carnivores) • consume secondary consumers and may or may not also consume primary consumers and primary producers, e.g. birds	
Third trophic level	Secondary consumers/second-order consumers (omnivores and carnivores) • consume primary consumers and may or may not also consume primary producers, e.g. skinks	
Second trophic level	Primary consumers/first-order consumers (herbivores and omnivores) • consume primary producers, e.g. caterpillars	
First trophic level	Primary producers • manufacture own food source through photosynthesis, e.g. plants and algae	Autotrophs
No trophic level	Decomposers and detritivores • consume and recycle dead plant and animal material, supporting the function of the ecosystem, e.g. fungi, bacteria, earthworms and flies	

Food chains

The movement of organic molecules and the energy contained within their bonds through the ecosystem can be illustrated diagrammatically in **food chains** and **food webs**. Food chains show a simple linear progression of matter and energy. Food webs illustrate the complexity of interactions in a particular community.

In the example beginning with the eucalypt leaf shown in Figure 4.1.13, the kookaburra feeds on the skink that feeds on insects that feed on the eucalypt leaves. In this food chain, the eucalypt is a producer. It captures the sun's rays for energy, so it is also in the first tropic level. Similarly, in the example beginning with algae, the catfish feed on the crustaceans, which feed on algae. Algae are the producers and are therefore also part of the first trophic level. The crustaceans and catfish are consumers, because they eat other living things to meet their needs for nutrients and energy.

The other organisms are all consumers. They eat other living things to meet their needs for nutrients and energy.

Almost all food chains start with energy from the sun. Plants (producers) use chlorophyll to capture this energy in order to produce their own energy via photosynthesis, which is then acquired by consumers when they eat the plants. A few food chains start with energy sources other than the sun, such as those around deep sea hydrothermal vents, in which bacteria use chemosynthesis rather than photosynthesis.

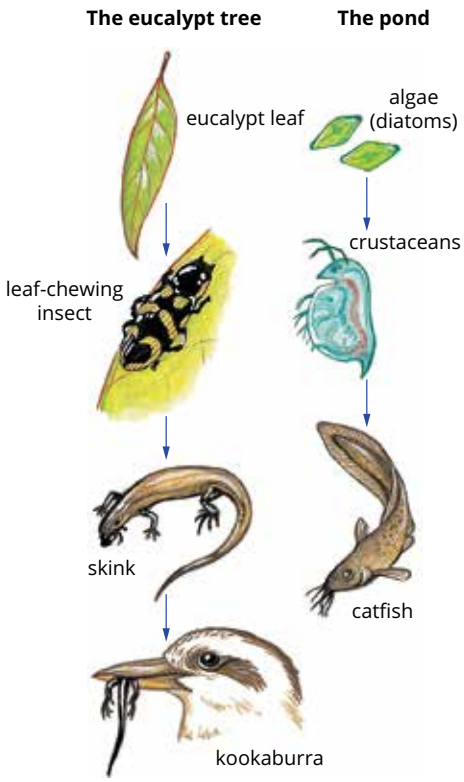


FIGURE 4.1.13 Two simple food chains: one in the eucalypt tree and one in the pond.

Each feeding level in a food chain is called a trophic level. Trophic levels are defined levels of energy transference, following the transfer of energy from consumption and absorption from one level to the next (Figure 4.1.14). All producers are in the first trophic level. Consumer organisms are at higher trophic levels. Herbivores are at the second trophic level, because they feed on the producers at the first level. Primary/first-order carnivores, feeding on herbivores, are at the third trophic level, and so on.

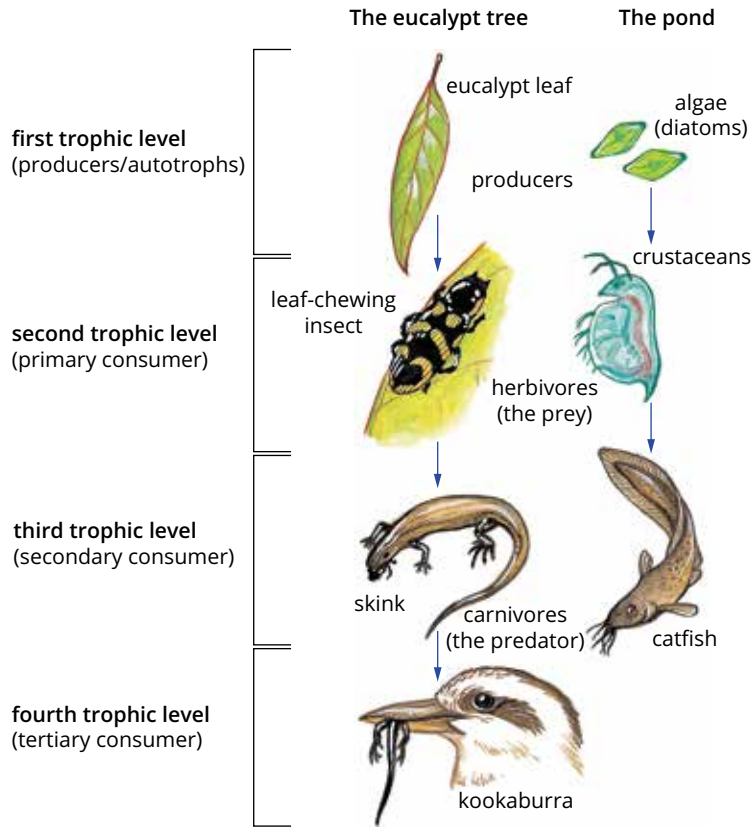


FIGURE 4.1.14 The trophic levels in food chains.

In a food chain, the direction of the arrows shows the direction of matter and energy flow. Food chains generally begin with a producer. In the example in Figure 4.1.14, the algae and the eucalypt tree are both producers. All of the other organisms are consumers. The arrows in food chains show several types of feeding relationships.

Predator–prey food chains

Many food chains are predator–prey food chains. A simple example from the pond in Figure 4.1.14 is the catfish that feeds on crustaceans that feed on algae. The food chain linking the kookaburra, skink, insect and eucalypt tree is also an example of a predator–prey food chain.

Predators usually eat prey smaller than themselves. Big fish eat little fish and a hawk preys on small animals like mice or rabbits. However, this is not always the case. For example, hyenas are smaller than the antelopes upon which they frequently prey. The flow and transfer of energy is easily observed from prey to predator.

Parasite–host food chains

Parasite–host food chains (Figure 4.1.15) look a little different from predator–prey chains. Parasite–host food chains have a large organism (the host) as a source of food for a smaller organism (the parasite). The transfer of energy from host to individual parasite is usually too small to notice; however, energy transfer from host to a population of parasites can be measured.

Detritivore and decomposer food chain

In temperate forests, only about 10% of the plant material is eaten directly each year by herbivores. Much of the plant material falls as leaf litter. Dead leaves, dead branches, fallen tree trunks, dead roots in the soil and the remains of dead animals and wastes such as faeces (called detritus) are a major source of food for detritivores, such as snails, worms, termites, springtails, millipedes and mites, and decomposers, such as fungi and bacteria. Detritivore and decomposer food chains are most abundant in forests and incorporate most of the energy flow and transfer (Figure 4.1.16).

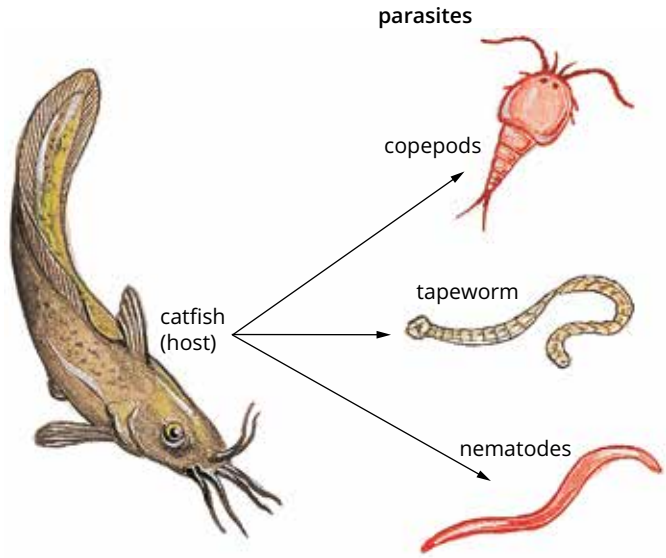


FIGURE 4.1.15 Parasite–host food chains in the pond. The food chains go from the larger host, the catfish, to smaller organisms, the parasites.

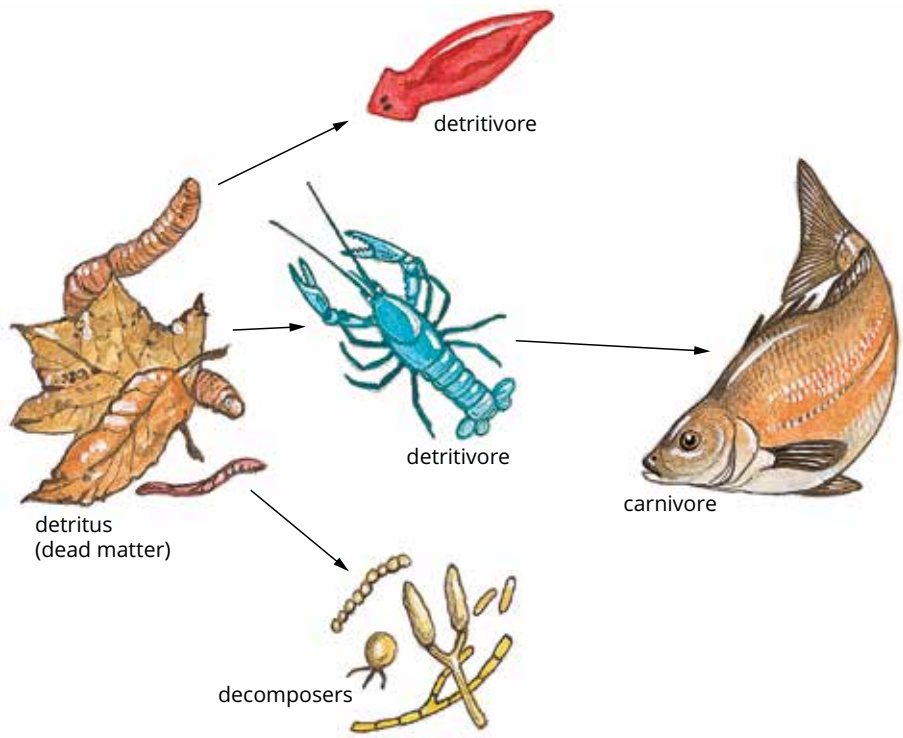


FIGURE 4.1.17 Detritivore and decomposer food chains in the pond both start with dead matter.

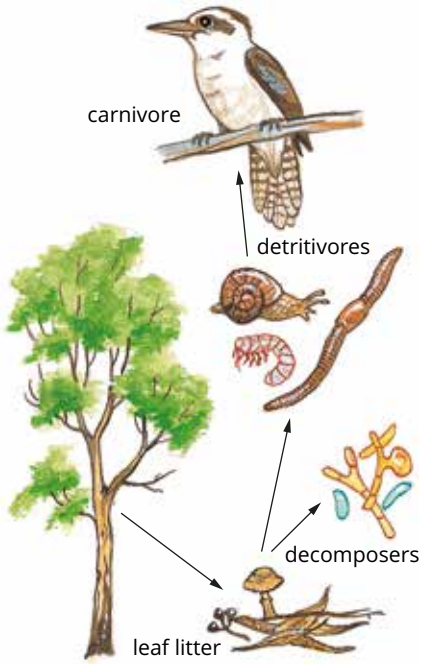


FIGURE 4.1.16 Detritivore and decomposer food chains are very important in terrestrial ecosystems, where plant litter accumulates on a forest floor.

Decomposers and detritivores are also important in aquatic ecosystems, where detritus builds up on the bottom of a pond, lake or bay (Figure 4.1.17).

As a detritivore eats dead leaves, it also eats decomposers (bacteria and fungi) that are on and in the dead material. The bacteria and fungi are sometimes the detritivore's main source of energy, because the detritivore does not digest all of the leaf material that it eats. Detritivores are important because they physically break down litter into small particles. They consume some of the stored energy in the leaf litter while other parts of the dead leaves are chewed and passed out of the animal as faecal pellets. These smaller particles are then easier for decomposers to consume.

Some detritivore animals also eat faecal pellets (either their own or those of other species).

The decomposers, bacteria and fungi are the last link in this chain of eating, re-eating and breaking down detritus (Figure 4.1.18). The digestive enzymes that decomposers secrete over their food break down the organic matter into soluble organic molecules (such as sugars and amino acids), which are absorbed and eventually turned into inorganic nutrients (such as carbon dioxide, nitrogen and phosphate), which are released as waste. The waste products are released into the environment, into the pond water, the soil and the air.

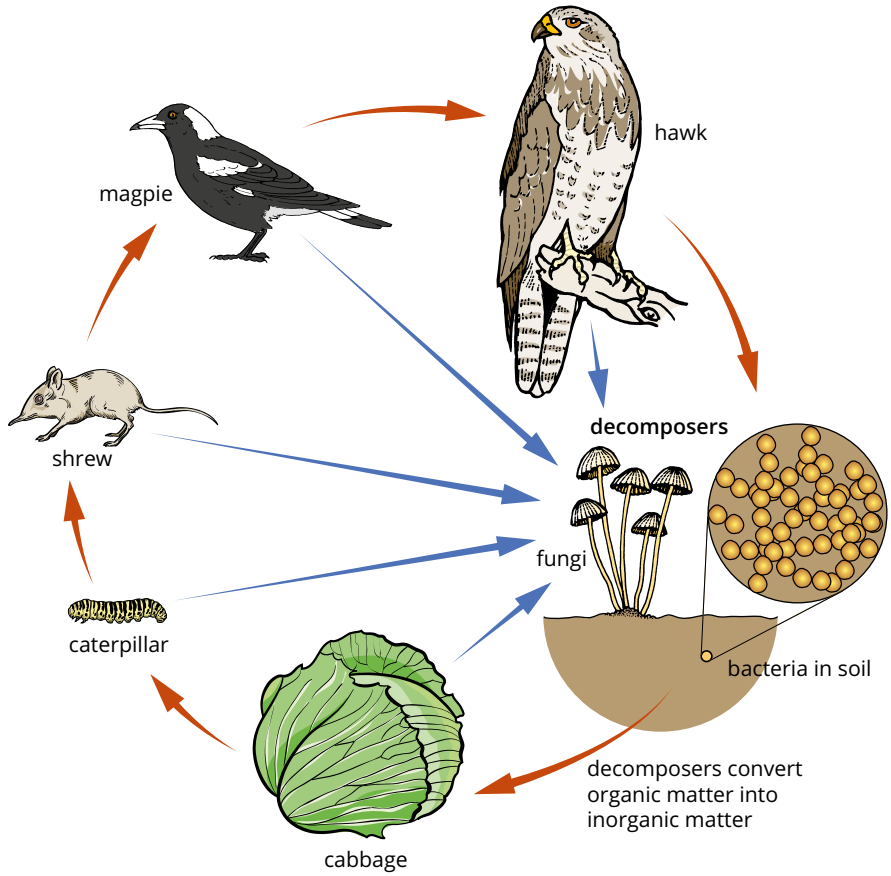


FIGURE 4.1.18 Decomposers complete the cycle of the breakdown of organic materials into simple inorganic molecules.

Food webs

Within one ecosystem there may be dozens or even hundreds of different food chains. These can all be linked together to form a food web. Most organisms eat more than one type of food, so food chains often overlap. There are numerous energy transfer pathways through an ecosystem. In the pond, the catfish feeds on small crustaceans and insects (Figure 4.1.19) as well as snails and small fish. The catfish is in at least two different food chains.

algae → *Daphnia* → catfish

and

algae → protozoan → *Daphnia* → water strider → catfish

In the first food chain, the catfish is at the third trophic level (and is a first-order carnivore and also a second-order consumer). In the second food chain, the catfish is at the fifth trophic level.

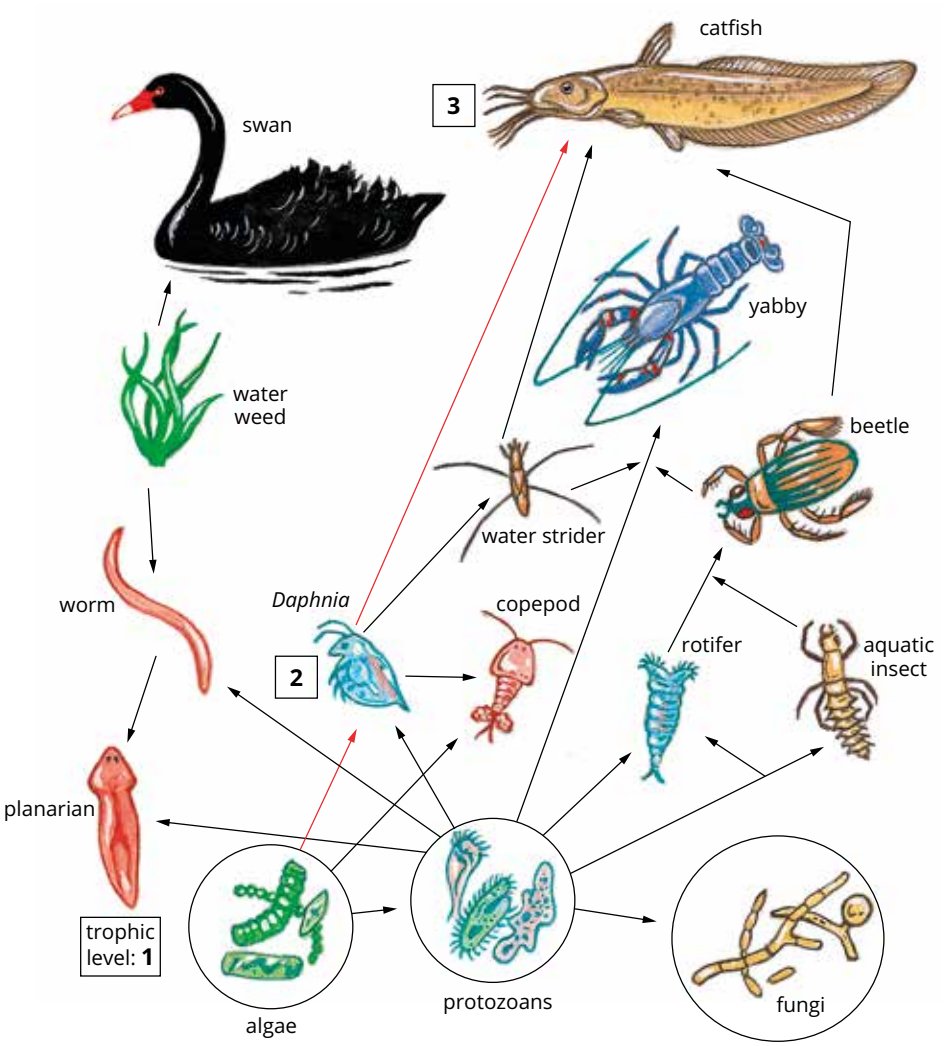


FIGURE 4.1.19 The food web of a pond. Food chains intertwine because most organisms eat more than one type of food. One food chain (red arrows) illustrates three different trophic levels.

The complexity of food webs tends to give ecosystems stability. In a simple food chain, the removal of one species can have a disastrous effect on all the organisms that follow it in the chain. The loss of one species from a complex web generally has less effect, since alternative food sources are available. Biodiversity is not just imperative to populations and survival, but also ecosystem stability.

Pyramids of biomass and energy

Plants convert 1–3% of the sun's energy falling on them into organic matter. Herbivores incorporate around 10% of the energy in the food they consume into biomass. Carnivores are more efficient at utilising their food intake. They generally convert around 20% of the food that they consume to biomass. Generally, throughout an entire food chain, the average transfer from one level to the next is 10%, although the actual transfer is quite variable and depends on the nature of the ecosystem and the time of the year.

Energy transfer can be illustrated using a Sankey diagram. In a Sankey diagram, inputs always enter from the left, unwanted or wasted outputs go down, and the useful or desired outputs exit to the right. The size of the arrow is proportional to the size of the input or output.

Figure 4.1.20 is a Sankey diagram that illustrates the fate of the energy from the sun falling on the Earth. It shows that only a very small amount of the light energy from the sun is captured by photosynthesis and transformed into chemical energy as the bonds between glucose molecules.

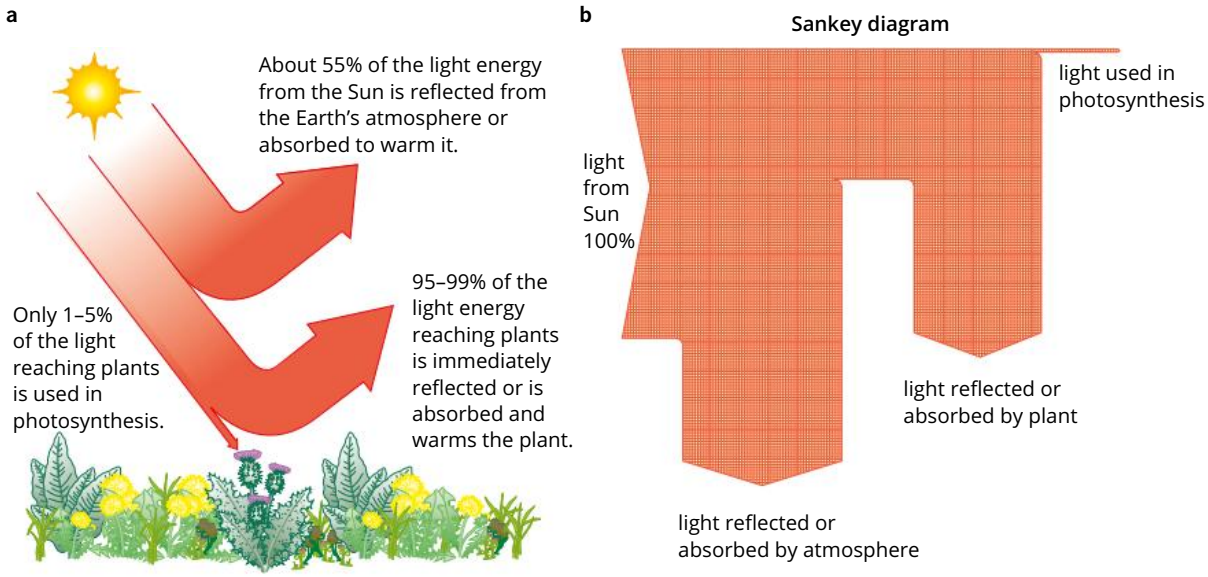
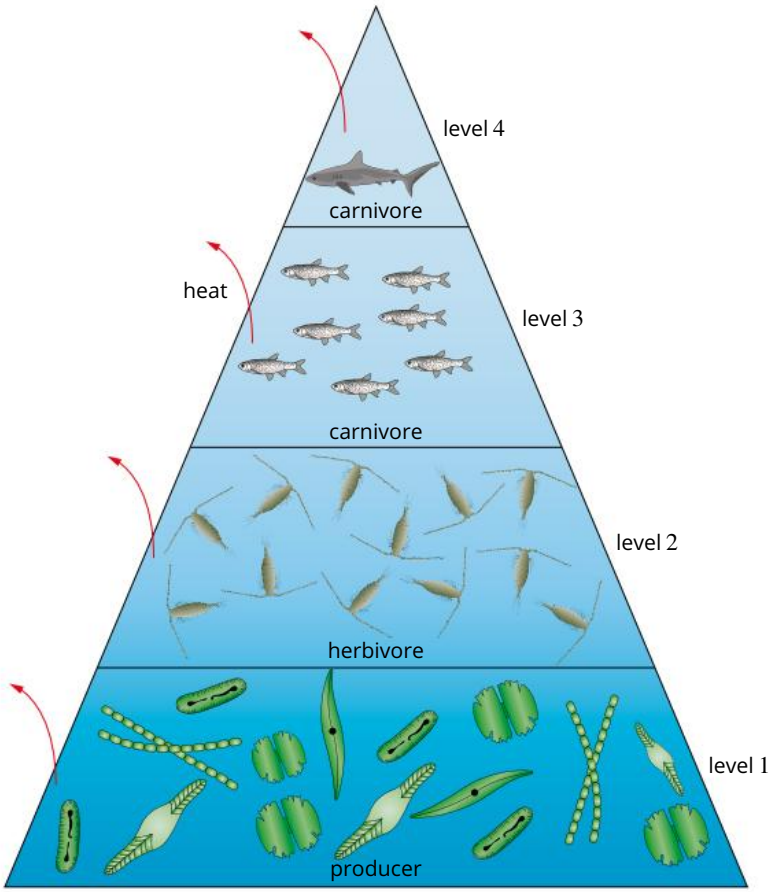


FIGURE 4.1.20 (a) A pictorial representation of the fate of light energy that falls on the Earth. (b) A Sankey diagram illustrating the fate of light energy falling on the Earth.



One way to measure energy flow along food chains is to measure biomass. The energy being transferred is chemical energy held by the bonds between the molecules of the chemicals, which form the biomass of the organisms. With each transfer of energy along a food chain, from producers to herbivores to carnivores, there is a decline in the amount of energy (and biomass) available for consumption. This is best shown as a diagram called an **ecological pyramid of energy** or ecological pyramid of biomass. The amount of stored energy in biomass available to higher-level carnivores at the top of the pyramid is very small compared with that available in the producers at the base. This decline in stored energy at each trophic level means that most food chains are usually limited in length to four or five trophic levels, as the average amount of energy transferred to the next level is 10%. For this reason, the top carnivores usually survive by eating a variety of foods. Also, the number of individuals in the highest trophic level is usually very small. This reduction in energy and numbers of individuals is illustrated in Figure 4.1.21.

FIGURE 4.1.21 A pyramid showing the decline in biomass and energy along a food chain through the trophic levels from producer to herbivore to top carnivore. In general, about 10% of the energy at one trophic level appears in the next. This decline limits the length of food chains.

The reason that so little energy is transferred between trophic levels is that much of the energy stored in an organism's food is used to power the metabolism of the organism. The energy is released from the food during the process of cellular respiration. The released energy is used to build ATP, which is an immediate energy source for the organism. Cellular respiration is a pathway that, like photosynthesis, involves many chemical reactions and enzymes. It can be summarised according to the following equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy as ATP}$$

Refer to Chapter 3 of *Pearson Biology 11 Queensland Student Book* for more information about cellular energetics.

In a particular ecosystem, most of the energy absorbed by producers is used by them for metabolic processes. Only a small fraction is used to create new biomass. When the producers are eaten by the herbivores, much of their biomass is not digested. It is eliminated as waste, primarily faeces. The digestion and absorption of food is called assimilation. Of the assimilated material, most is used in metabolic processes, especially respiration, and is largely lost to the surrounding environment as heat. It is therefore not available to the next trophic level (Figure 4.1.22). The only energy that can be transferred to the next trophic level is the energy contained in biomass.

In most ecosystems, the biomass decreases from one trophic level to the next. However, pyramids of biomass are snapshots of a moment in time. They can show inversion, where one of the prey species has a high reproductive rate but the predator species has a slow growth rate. For example, at a particular moment in time in one area, the mass of whales may exceed the mass of krill but this imbalance does not persist. Krill grows, breeds and dies at a fast rate, so their overall biomass increases rapidly, but whales grow very slowly and live for many years.

Blue whales typify this relationship between krill and their predators. Blue whales reach a mass of 140 000 kg by the time they are fully grown, but this can take more than 80 years. Growth rates after weaning average 3.9 kg per day or 0.006% increase in mass. Krill, on the other hand, have a life span of around 6 years and reach full size in a few months. The krill population of the Antarctic region is huge and has a gross production rate of 1.27×10^9 kg d^{-1} during their period of growth. This represents an increase in biomass of approximately 1% per day.

Today, the mass of krill at any moment in time exceeds that of the predators which feed upon it, but this was not the case in the past. For example, there are just 12 000 blue whales in the world today but before whaling became a major industry the number is thought to have exceeded 350 000. Most other krill-dependent whales and large marine animals have experienced similar declines due to human activities. A more typical pyramid of biomass of the Antarctic is shown in the pyramid in Figure 4.1.23.

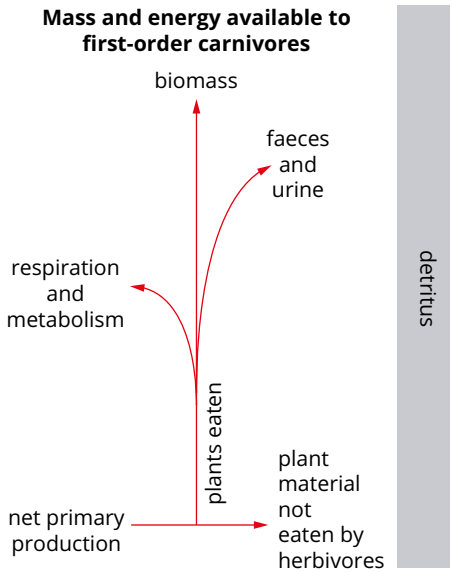


FIGURE 4.1.22 The fate of material and energy captured during net primary production.

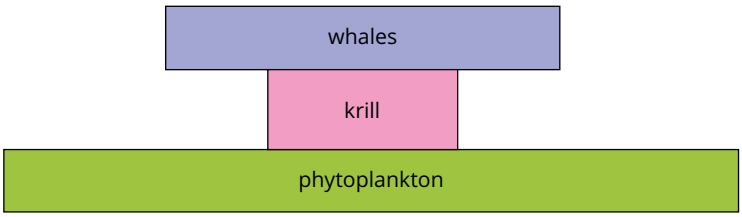


FIGURE 4.1.23 A diagrammatic representation of the expected pyramid of biomass involving Antarctic whales taken at one moment in time.

A detailed study of the movement of biomass and energy through the trophic levels of an ecosystem was undertaken at Silver Springs in Florida by H.T. Odum in 1957. This long-term study used a variety of methods, including measuring fish respiratory rates and the rate of weight gain of captive snails, to calculate growth and tissue utilisation rates in order to develop pyramids of biomass and energy for the Silver Springs lake ecosystem.

Figure 4.1.24 shows the pyramid of biomass calculated by Odum for this ecosystem. These pyramids are hard to generate accurately because there are many variables to consider. Growth rates of organisms can be affected by many factors, including temperature and climate, pollution and disease, all of which can be both quite variable and random in their impacts.

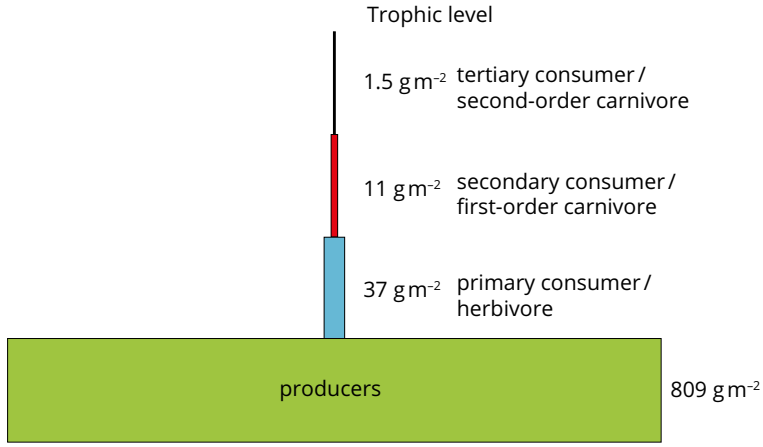


FIGURE 4.1.24 The pyramid of biomass for the Silver Springs lake ecosystem. Biomass is calculated as grams per square metre of dry weight.

The pyramid of energy for this ecosystem shows how significantly the energy available to organisms in higher trophic levels declines (Figure 4.1.25). It also explains why very few food chains exceed four or five links.

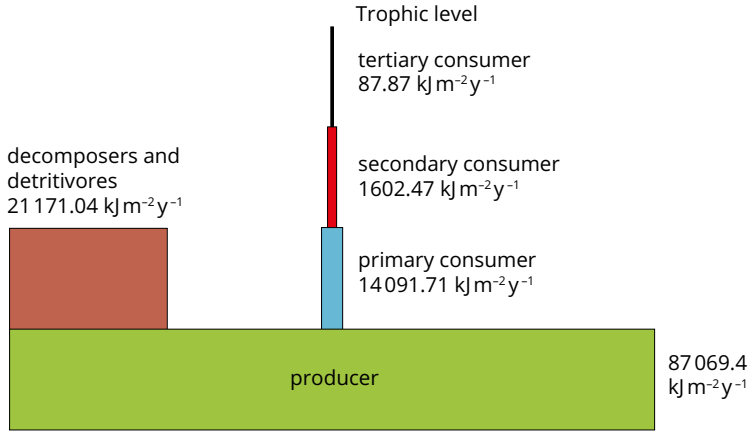


FIGURE 4.1.25 The pyramid of energy for the Silver Springs lake ecosystem. Energy is calculated as kilojoules per square metre per year.

Within ecosystems, matter is constantly recycled. As organisms produce wastes or die, detritivores and decomposers break down the organic matter into its constituent molecules. These molecules can then be used by other organisms such as plants and bacteria as the raw materials for building more complex substances. In contrast, energy is not recycled. All of the energy that is initially captured during photosynthesis is eventually lost back into the environment as heat.

CYCLING OF NITROGEN, CARBON AND WATER THROUGH ECOSYSTEMS

The building blocks of living things are compounds such as carbohydrates, lipids, nucleic acids and proteins. These compounds are rich in carbon, hydrogen, oxygen and nitrogen. These elements are recycled constantly through ecosystems in a process called **biogeochemical cycling** or nutrient cycling. This cycle involves animals, plants and bacteria, along with decomposers and the non-living surrounds of the organisms.

Cycling of nutrient is essential. Without cycling, molecules would eventually all be trapped in the dead bodies and waste materials of once-living things.

Water

Water moves constantly through the environment, continually cycling through a series of events and processes. It is incorporated into carbohydrates during photosynthesis and released into the air during respiration and decomposition. Water is also moved through the ecosystem by the processes of evaporation, precipitation and transpiration (Figure 4.1.26).

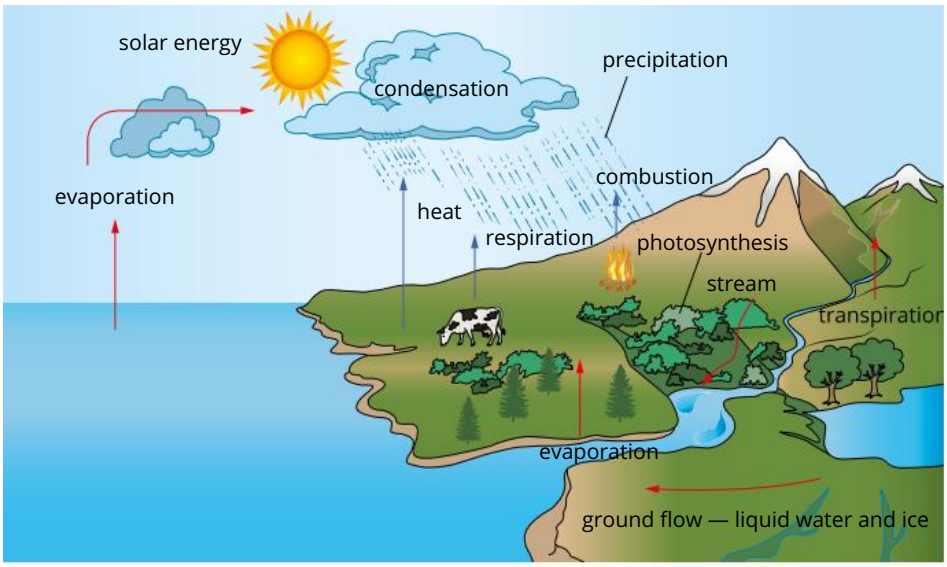


FIGURE 4.1.26 The water cycle is a complex interplay between the land, sea and atmosphere.

Most of the water on Earth (around 95.6%) is held in the oceans. Energy from the sun causes water to evaporate from the oceans and form water vapour in the atmosphere. Winds move the water-laden air to other parts of the planet where cooling of the air results in precipitation of the vapour as rain, hail or snow. Most of the precipitation falls back into the oceans but some falls on the land. Of the precipitation that falls on the land, some re-enters the air as a result of the evaporation of liquid water or sublimation of ice and snow. Finally, ice and snow can be compacted, as at the ice caps and glaciers, and the water can remain locked there for a period ranging from days to thousands of years.

Water may also run off over the surface of the land. As snows melt or rain falls, the water flows over the ground. Much of this water ends up in lakes and streams and, due to gravity, eventually flows into the sea and returns to the oceans. Some of the water percolates (filters gradually) down through the soil to form groundwater or supply aquifers (underground caverns). Plants absorb groundwater to use in photosynthesis but most of the water absorbed through their roots is lost to the atmosphere as a result of transpiration (see Chapter 5 of *Pearson Biology 11 Queensland* for more information about transpiration). The rest of the groundwater eventually either percolates into rivers or streams or the ocean. Some of the groundwater collects in aquifers, which may store the water underground for many thousands of years.

FIGURE 4.1.27 The extent of the Great Artesian Basin under northern Australia.

Much of Queensland is sitting over a large underground aquifer that many rural families and towns tap into with wells for fresh water. The aquifer, called the Great Artesian Basin, is thought to be the largest aquifer in the world. It lies under most of the north-eastern part of Australia (Figure 4.1.27).

The water in the Great Artesian Basin was mostly collected over the last 2 million years. Currently, the extraction of water for agriculture and industry exceeds the amount entering the basin as ground percolation.

The global water cycle as measured by NASA

Using data gained from Earth-observing satellites, hydrologists at NASA have calculated the amounts of water involved annually in the water cycle of the Earth.

They have estimated that 449 500 km^3 of water evaporates from the ocean each year. (For reference, the largest reservoir in Queensland, the Wivenhoe Dam, contains 1.165 km^3 of water.) On land, 70 600 km^3 becomes vapour due to transpiration, evaporation and respiration. This water collects in the atmosphere and is blown to other parts of the Earth, where it falls as precipitation.

Approximately 403 500 km^3 of water falls back into the sea, and 116 500 km^3 falls on the land as a result of precipitation. The fate of the water that falls on the land is estimated to be 45 900 km^3 entering rivers and streams that run into the oceans and 70 600 km^3 evaporating into the atmosphere. Ultimately, although it may take millions of years for a particular water molecule, all water ends up back in the sea.

Carbon

The carbon cycle is an important geochemical cycle which moves one of the important components of organic compounds (Figure 4.1.28). Carbon is stored in the atmosphere as carbon dioxide and in the ground as carbonates and fossil fuels. Decomposition, weathering of rocks and burning of fossil fuels releases carbon from the ground.

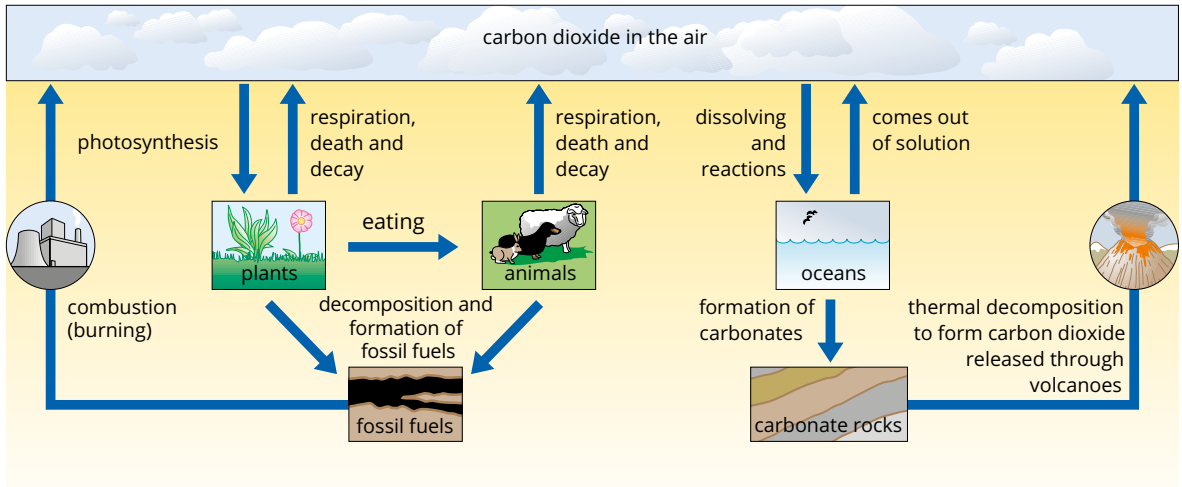


FIGURE 4.1.28 The carbon cycle.

Large amounts of carbon are sequestered (removed and stored) by the oceans, both dissolved in the water and incorporated into shells and skeletons of sea creatures and buried under sediment when they die.

Shells of most sea creatures are made of calcium carbonate ($CaCO_3$), which is one way of trapping carbon. Calcium carbonate is also formed when silicates in rocks weather, according to the equation:

$$CaSiO_3 + 2CO_2 + 2H_2O \rightarrow CaCO_3 + SiO_2 + CO_2 + 2H_2O$$

This equation shows that for each molecule of calcium silicate ($CaSiO_3$) weathered, one molecule of carbon dioxide is captured.

Estimates of the mean amounts of carbon in each part of the carbon cycle are difficult to make and different researchers have produced different values. Values are also revised as new data is generated. One set of estimates is shown in Table 4.1.2.

TABLE 4.1.2 Sources and fate of carbon on Earth

Carbon source	Absorbed from or released into the atmosphere, or in medium/long-term storage	Billions of metric tonnes per year
in the atmosphere	long-term storage	720
volcanoes	release	0.1
burning of fossils fuels	release	5–6
soil	long-term storage	1500
ocean water	long-term storage	39 000
fossil fuels	long-term storage	4000
weathering and erosion	absorbed and then stored	0.6
photosynthesis	absorbed	120
stored in plants	medium-term storage	560
respiration	release	120
marine sediments and sedimentary rocks	long-term storage	100 000 000

While a large part of the carbon cycle is geological in nature, living things also play a role. Plants and animals trap the carbon in fossil fuels. Plants also absorb carbon from the atmosphere and assimilate the carbon during photosynthesis. Animals eat the plants and incorporate the carbon from them into their bodies. When the animals and plants die, decomposers and detritivores release the carbon back into the environment as carbon dioxide.

Nitrogen

The nitrogen cycle is complex. It involves chemical, geological and biological processes. Animals, plants and several types of bacteria are involved. Air is around 79% nitrogen but gaseous nitrogen cannot be used by most living organisms. Plants must absorb their nitrogen from the soil as nitrates and animals obtain their nitrogen from plants or other animals. Nitrogen is an essential component of proteins and nucleic acids, so all organisms require a supply of it.

Bacteria play an important role in the nitrogen cycle as they are involved in making nitrates and also in the decomposition of nitrogen-containing compounds. Geological processes such as lightning are also involved. During electrical storms, nitrogen and oxygen react in the atmosphere to form nitrogen dioxide. When nitrogen dioxide dissolves in water, for example in rain drops, it forms nitrates. The nitrates are then washed from the sky into the soil where they can be absorbed by plants and used to build organic molecules such as proteins (Figure 4.1.29 on page 152).

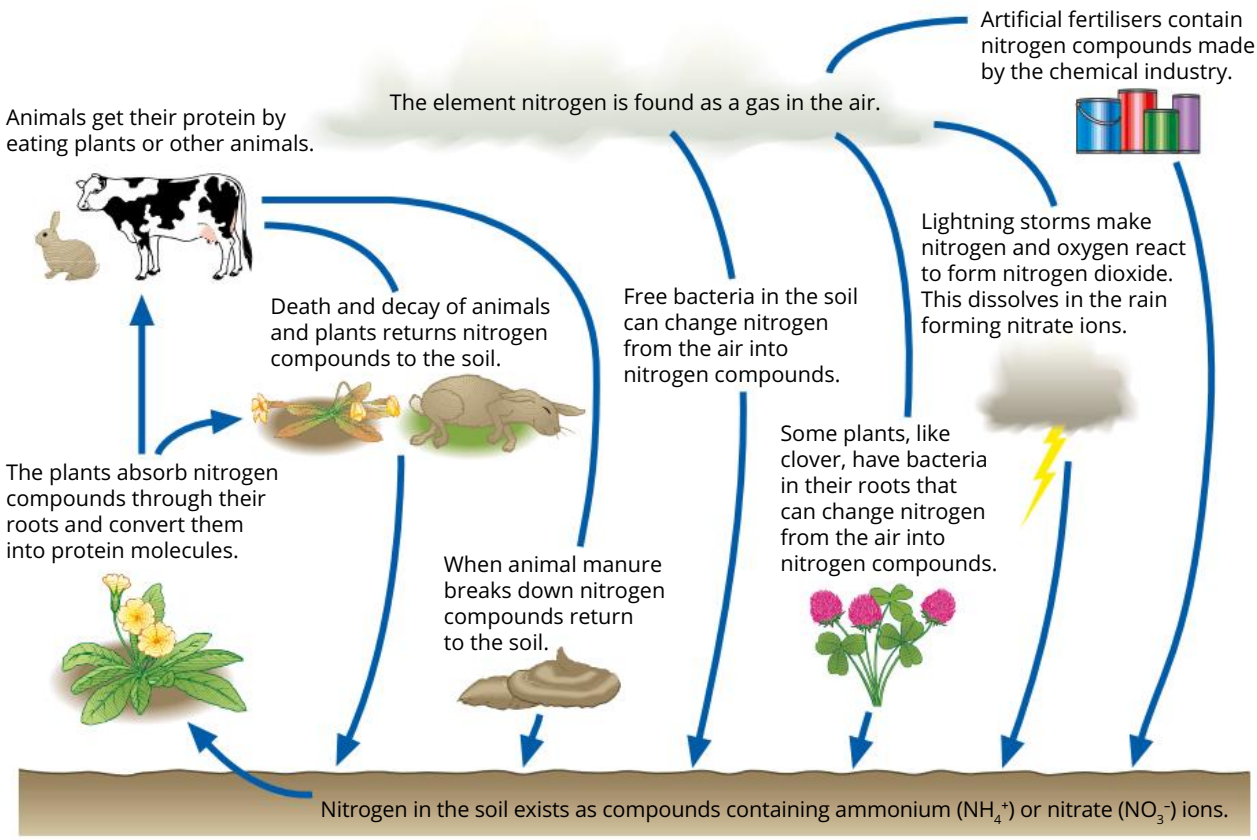


FIGURE 4.1.29 The cycling of nitrogen through the environment.

The nitrogen cycle involves four types of bacteria:

1 **Saphrophytic soil bacteria** decompose dead organic remains and turn them into ammonia (ammonification).

2 **Nitrifying bacteria** live in the soil and turn ammonia into nitrates (nitrification).

3 **Nitrogen-fixing bacteria** can live in the soil or in root nodules. Root nodules are swellings on the roots of a plant belonging to the pea family. These turn nitrogen gas into nitrates.

4 **Denitrifying bacteria** in waterlogged soil turns nitrate into nitrogen gas (denitrification).

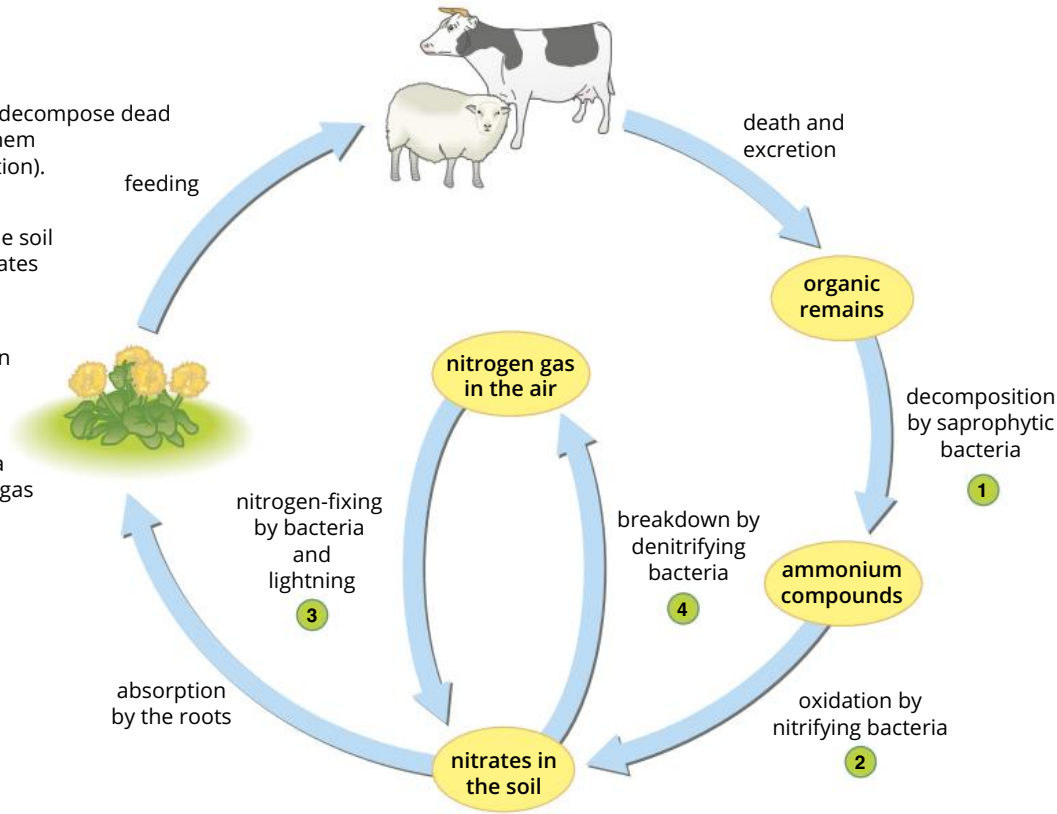


FIGURE 4.1.30 The roles of the four groups of bacteria in the nitrogen cycle.

Without bacteria, nitrogen would accumulate in the soil indefinitely and would eventually become unavailable to living organisms.

Four main groups of bacteria are essential to the cycling of nitrogen (Figure 4.1.30). Decomposing or **saprophytic bacteria** break down bodies and wastes to produce ammonia, **nitrifying bacteria** turn ammonia into nitrates, **nitrogen-fixing bacteria** trap nitrogen gas from the air and incorporate it into nitrates in the soil and **denitrifying bacteria** convert the nitrates in the soil back into nitrogen gas and allow it to be released back into the atmosphere.

Most of the bacteria involved in the cycling of nitrogen live free in the soil, but several species of nitrogen-fixing bacteria have developed a relationship with leguminous plants, such as peas and beans. These bacteria live in structures called nodules that form in the roots of the legumes (Figure 4.1.31). The plants supply the bacteria with sugars that they have produced during photosynthesis and the bacteria supply the plants with nitrates.

The process of turning organic material into ammonia is called **ammonification**.

The process of turning ammonia into nitrates is called **nitrification**.

The process of turning nitrate into nitrogen gas is called **denitrification**.

FIGURE 4.1.31 Soybean plants with root nodules that appear as small lumps attached to the roots. The nodules contain bacteria that absorb nitrogen from the air and produce nitrates that are then available to the plant.

The importance of nitrogen-fixing bacteria to plant productivity is critical. Free soil bacteria have been shown to fix around 0.625 $gm^{-2}\ y^{-1}$. However, when the bacteria are encapsulated in root nodules, their ability to fix nitrogen increases tenfold to around 6.25 $gm^{-2}\ y^{-1}$ in uncultivated grasslands and forests. In agricultural fields planted with clover or other legumes, where water and other nutrients are supplied by farmers, nitrogen-fixing bacteria in root nodules of the clover can increase the concentration of nitrogen in the soil in the form of nitrates. Studies have shown that crop production can increase this concentration to as much as 31.5 $gm^{-2}\ y^{-1}$. This is important to farmers as the use of nitrogen-fixing bacteria reduces their reliance on artificial fertilisers.

WS 3.2.4

4.1 Review

SUMMARY

- Autotrophs are organisms that synthesise their own complex organic molecules using simple substances like carbon dioxide and water.
- Most autotrophs (producers) use light as an energy source to build organic molecules.
- The rate at which producers convert light energy to chemical energy is called primary productivity. Matter that accumulates is called biomass.
- Heterotrophs (consumers) obtain their nutritional and energy requirements by consuming other living things.
- Consumers include herbivores, carnivores, omnivores and parasites (which consume living organisms), along with scavengers, detritivores and decomposers (which consume dead organisms).
- Bacteria and fungi, as decomposers, break down dead organisms and organic wastes to organic molecules and mineral nutrients.
- Food chains show the feeding relationships between organisms and flow of energy.
- Food chains link together into food webs, giving an ecosystem its stability.
- There are different types of food chains (predator–prey, parasite–host, detritivore and decomposer), depending on the relationships between organisms.
- Each feeding level in a food web is called a trophic level. An organism may function at more than one trophic level in a food web.
- The decline in energy and biomass moving up a food chain can be represented diagrammatically by an ecological pyramid.
- Because energy is lost (primarily as heat) in a food chain, energy and biomass decline at each trophic level and food chains are limited in length.
- Nutrient or biogeochemical cycles involve the transfer of chemicals from air, water and soil to food chains and back again. The rate of input and output is usually in balance.
- On a global scale, water cycles between oceans, the atmosphere and the land. On a local scale, water tends to flow through ecosystems rather than cycling.
- Carbon is stored in the atmosphere as carbon dioxide and as fossil fuels in the Earth. It is taken into food chains by photosynthesis and released during cellular respiration and decomposition.
- Nitrogen is stored as nitrogen gas (N_2) in the atmosphere. The nitrogen cycle is totally dependent on certain bacteria for both fixing nitrogen (ammonification and nitrification) for input into food chains and for releasing it back into the atmosphere (denitrification).
- Some nitrogen-fixing bacteria live symbiotically in the root nodules of plants such as legumes (e.g. peas).

KEY QUESTIONS

Retrieval

1 State the balanced summary equation for photosynthesis.

2 Describe the role of denitrifying bacteria in the nitrogen cycle.

3 Identify which of the Earth's biomes is the most productive:

a per square metre

b overall

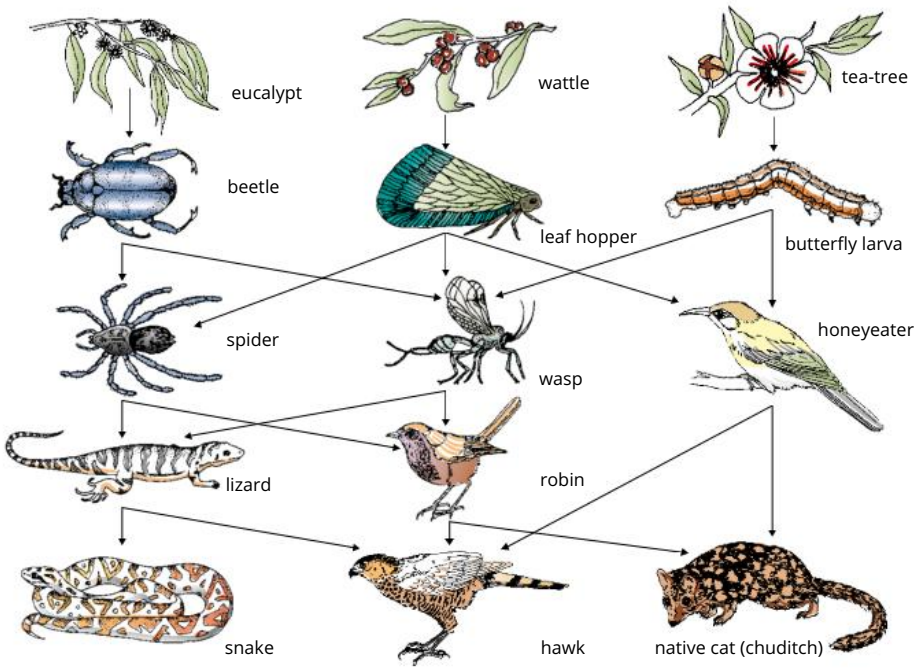
Comprehension

4 Explain how a decomposer is different to a detritivore.

5 Examine the food web at right.
   a Identify a producer in the food web.
   b Identify a second-order consumer.
   c Construct a simple food chain that finishes at the fifth trophic level.

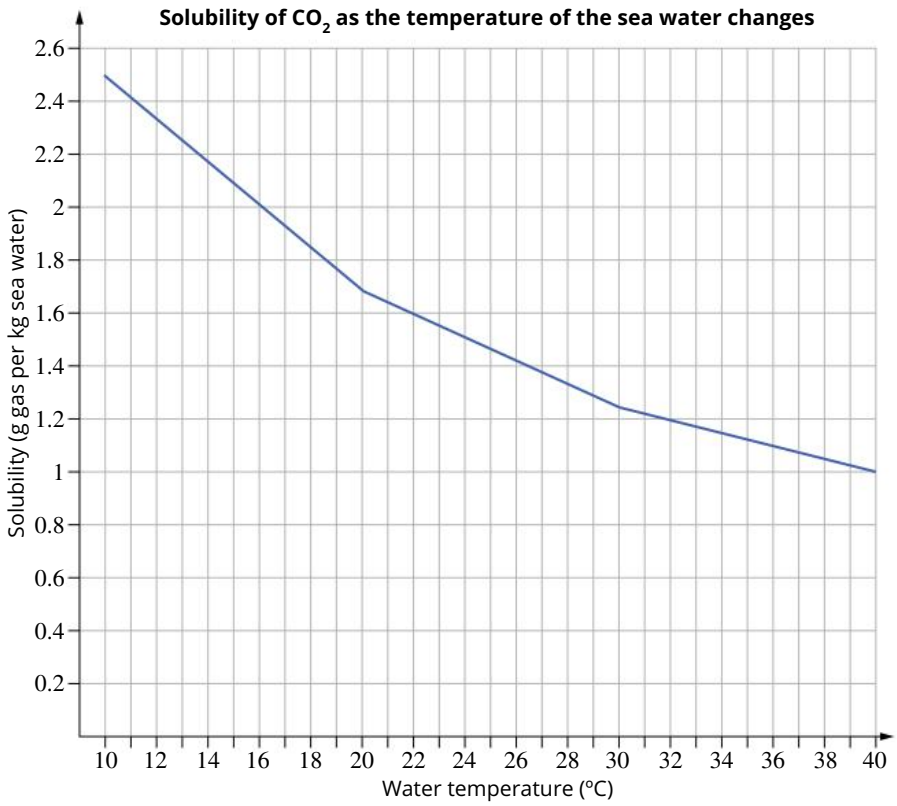
6 Explain why food chains are rarely longer than five trophic levels.

7 Calculate the number of glucose molecules that would be produced if 24 carbon dioxide and 24 water molecules were available for photosynthesis.



Analysis

8 The graph to the right shows the effects of temperature on the solubility of CO_2 in sea water. Mean annual seawater temperature is approximately 16°C. Calculate how many tonnes of CO_2 a rise of 1°C would release into the atmosphere if the oceans are saturated and currently hold 39 000 billion metric tonnes of CO_2.



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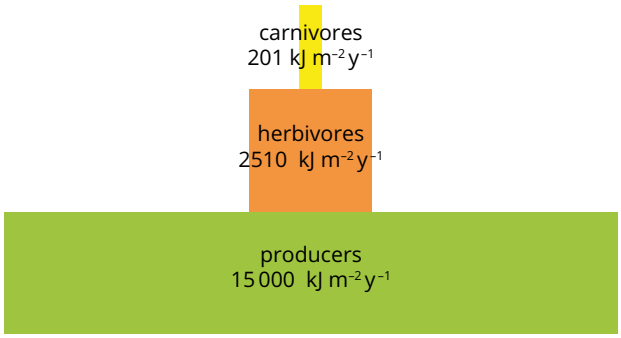
4.1 Review *continued*

9 A food pyramid is shown below for a marine ecosystem.



Calculate the percentage transfer of energy between the trophic levels. Give your answers to two decimal places.

10 Rice is a plant that uses the C_4 photosynthetic pathway while corn (maize) uses the C_3 photosynthetic pathway. Deduce which of the two plants is likely to result in higher crop productivity.

11 The following is a food chain for the Australian fur seal.
phytoplankton → krill → small fish → squid → Australian fur seal → great white shark

The average male fur seal has a mass of around 300 kg.
Calculate the number of kilograms of phytoplankton contributed to the biomass of the fur seal. Use the 10% rule for your calculation.

12 An ecologist reported that the primary productivity in a pond she was studying changed from winter to summer. Contrast the expected productivity during winter and summer. Justify your proposals.

13 Many lawns are a mixture of grass and clover. Determine the advantage of planting clover.

14 Consider a simple ecosystem consisting of a single food chain where a crop plant is eaten by an insect (such as a grasshopper), which is then eaten by a bird (such as a kestrel).

a Assess what will happen to the number of grasshoppers if the kestrels are killed.

b Predict the subsequent effect on the crop.

c Compare the effect of shooting one species of bird, if the ecosystem were more complex with more food chains cross-linked in a food web, to the situation of the simple food chain.

4.2 Functioning ecosystems

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand the concept of niche
- understand the principle of competitive exclusion and niche partitioning
- describe the beneficial, harmful and benign relationships between species; including amensalism, commensalism, mutualism, parasitism and predation
- explain the concept of a keystone species
- use data to identify likely keystone species within a particular ecosystem.

This module considers the organisational levels that ecologists use to study ecosystems. It examines the relationships organisms have with each other and their non-living surroundings and the consequences of disruptions to those relationships through natural and artificial causes.

NICHE

The type of place where an organism lives is its **habitat**. It is the 'address' where you would go to find the organism. The **niche** of an organism is the role and space an organism fills in an ecosystem, including all its interactions with the biotic and abiotic factors of its **environment**. In simple terms, this is how it operates in its habitat. A habitat could be a river, a tree or a desert, but a niche is how the organism uses the resources available in its habitat. The niche includes what the organism eats, where it sleeps, when it sleeps and takes into account all of the adaptations of the organism.

Ecological niche

The concept of ecological niche is complex and ill-defined, despite being revised and developed over time. At its most basic, an **ecological niche** is how and where an organism lives and interacts with other organisms and the abiotic environment. Put another way, the niche is the full range of resources an organism uses and how it uses them, along with the environmental conditions it can tolerate and the interactions it has with other species. The ecological niche of a population of organisms is the culmination of all of the adaptations of that population that have occurred through natural selection, including structural, behavioural and physiological adaptations (refer to Chapter 9 for more information on adaptations).

A niche in operation: Tasmanian devil

The Tasmanian devil (*Sarcophilus harrisii*) has been the subject of much research as it is highly endangered. Studies have looked at how adults and juveniles interact with their environments.

Adult Tasmanian devils are the only specialised mammalian carnivorous scavengers in Australia. They eat a broad range of foods. They scavenge invertebrates, birds and fish as well as the bodies of other animals. They perform a vital function by removing the remains of dead animals from the habitat (Figure 4.2.1). This assists with the cycling of nutrients and reduces the number of decomposing carcasses, which may encourage disease or parasites, especially blowfly maggots. Since the introduction of cars, devils have taken advantage of road kill; however, this increases the chances of them being hit by a car themselves. Adult devils also hunt live animals such as small mammals. They are solitary hunters that prey on weak or injured prey, culling the less fit members of the prey species. Adult devils are nocturnal. During the day, they hide in dense bush or in dens that have been dug out of the ground. The dens may be old wombat burrows, hollow logs or caves. Adult Tasmanian devils have no natural predators.

FIGURE 4.2.1 Tasmanian devils feeding on a kangaroo carcass.

Juvenile Tasmanian devils occupy a different niche. They are more likely to climb trees and forage in the branches, where they eat insects, small mammals, birds' eggs and small lizards. They are at least partly diurnal, which reduces their competition with the larger and more aggressive adults, but brings them into competition with spotted-tail quolls. Adult spotted-tail quolls also prey upon juvenile devils, as do owls and eagles.

Fundamental niche versus realised niche

Most ecologists recognise two aspects to the niche of an organism. The **fundamental niche** is the theoretical potential use of resources for a particular species, while the **realised niche** represents the actual use of resources (Figure 4.2.2). In the absence of any competition for resources, the fundamental and actual niches are the same, but this is rarely the case.

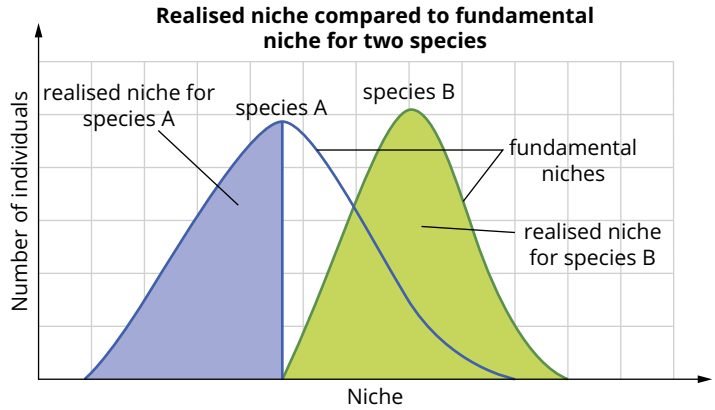


FIGURE 4.2.2 The realised niche of an organism is the actual use of resources whereas the fundamental niche is the potential use. The realised niche for species B is the same as its fundamental niche whereas the realised niche for species A is smaller than its fundamental niche. The realised niche of species A does not overlap that of species B, resulting in niche separation.

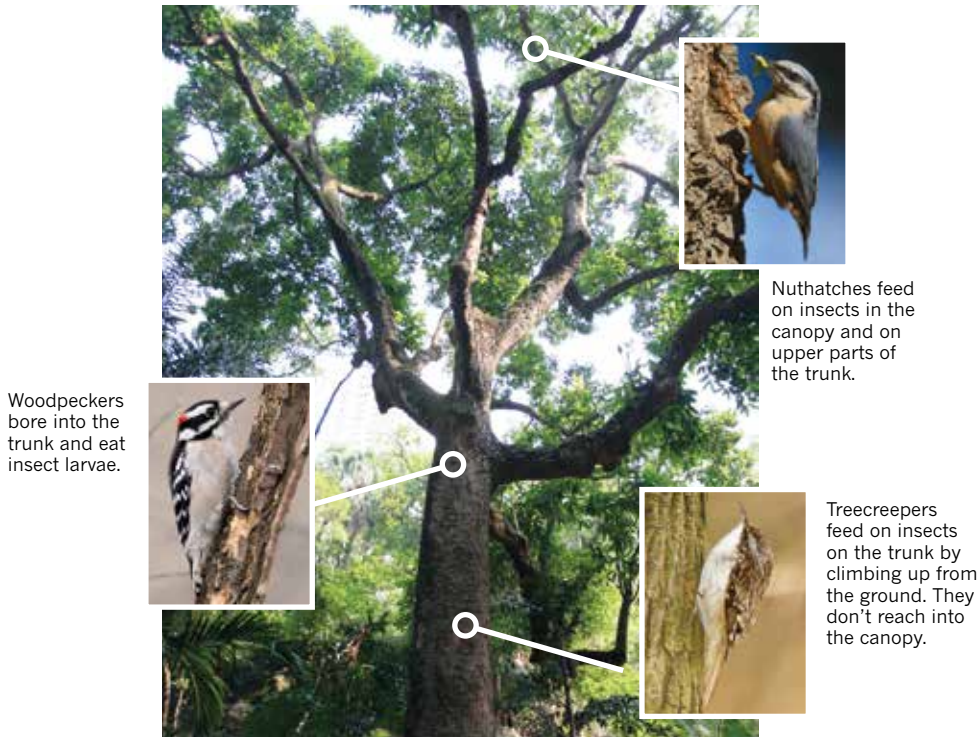


FIGURE 4.2.3 Nuthatches, brown tree creepers and woodpeckers feed on the same tree but do not compete for the same resources, so they occupy different niches.

While many species can occupy the same habitat, the principle of **competitive exclusion**, also called Gause's law of competitive exclusion, states that two species requiring the same resources cannot exist in the same niche. Species can only coexist if they have different requirements. For example, nuthatches, brown tree creepers and woodpeckers all feed on insects on the same trees, but their niches do not overlap (Figure 4.2.3). The nuthatch feeds in the canopy, the creeper feeds on insects on the tree trunk, and the woodpecker bores into the tree trunk and feeds on the insects and larvae it finds there. The three species use the resources of their environment differently and therefore occupy different niches.

Competitive exclusion leading to a difference in the realised niche from the fundamental niche is best illustrated by the classic study of the limpets undertaken by Joseph Connell along the coast of Scotland. He examined competition between two species of barnacle living in the intertidal zone (Figure 4.2.4).

Two barnacle species live along the rocky shore of Scotland: the recently reclassified *Semibalanus balanoides* and *Chthamalus stellatus*. The larvae of both species are free swimming but become sessile (stationary) when they become adults. The adults of both species attach to rocky outcrops but the two species do not grow mixed together. Instead, they are stratified with *C. stellatus* growing in a strip above *S balanoides*. Connell investigated the relationship between the two species to identify why the two species did not mix.

Connell chose one area where the two species were growing (Figure 4.2.5).

FIGURE 4.2.4 Barnacles growing on rocks in the intertidal zone.

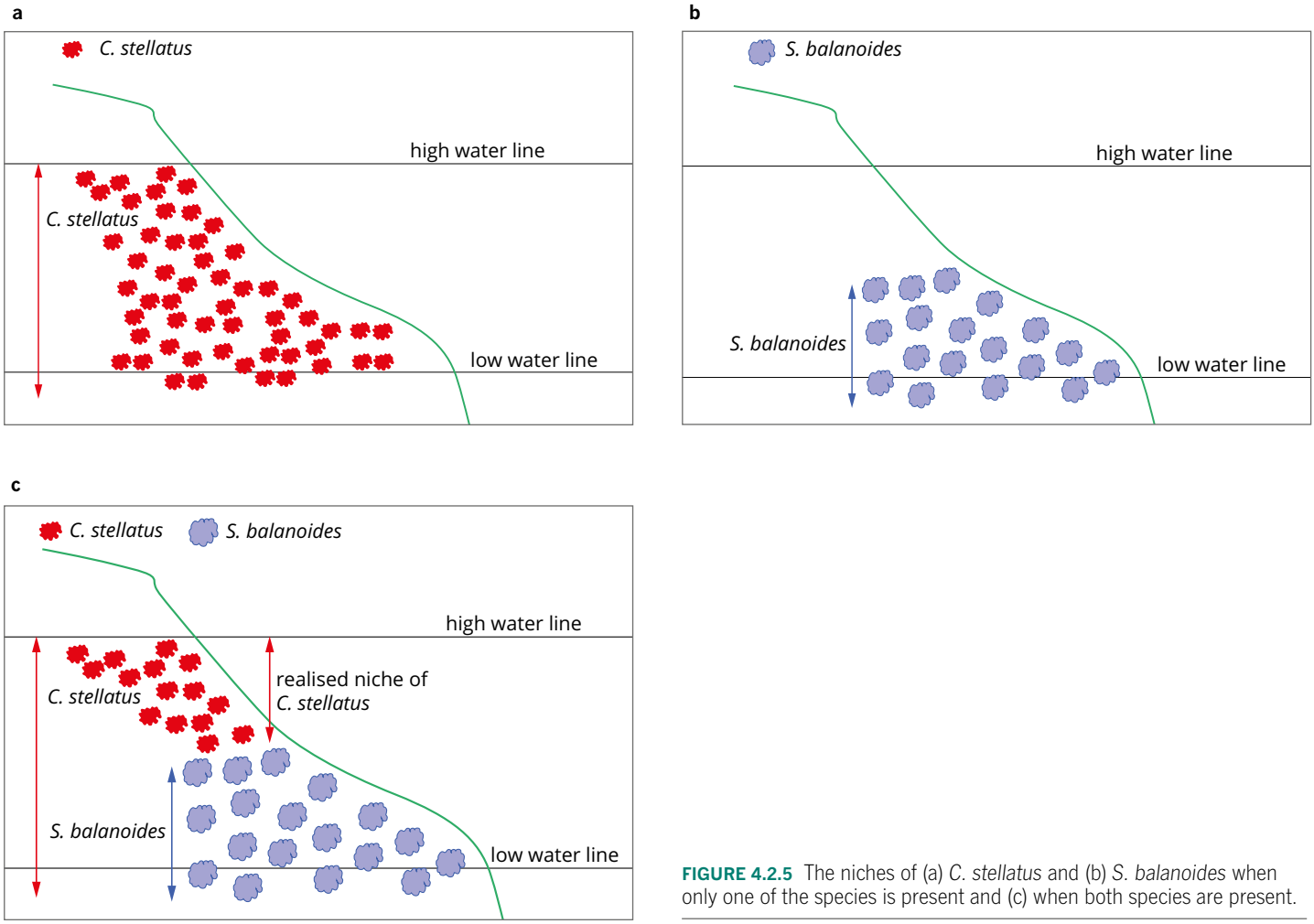


FIGURE 4.2.5 The niches of (a) *C. stellatus* and (b) *S. balanoides* when only one of the species is present and (c) when both species are present.

Initially, Connell removed the *C. stellatus* so the *S. balanoides* could grow without competition. Despite the removal of the *C. stellatus*, the *S. balanoides* did not extend their range. Connell hypothesised that the *S. balanoides* was unable to cope with the extended periods of exposure to the air which occurred during low tides. Connell then inferred that the realised and fundamental niches of *S. balanoides* are the same.

Connell then undertook a second experiment where he removed the *S. balanoides* so that the *C. stellatus* could grow without competition. In this case, the range of the *C. stellatus* increased, with the *C. stellatus* moving down to also fill the space left by the removal of the *S. balanoides*. Connell's conclusion was that, in areas where the two species coexisted, the *S. balanoides* was outcompeting the *C. stellatus*. As a result, the realised niche of the *C. stellatus* was smaller than its fundamental niche, due to the competition.

The accommodations undertaken by species that result in species having realised niches that do not overlap is called **niche partitioning**. Niche partitioning means that species are not competing for resources.

INTERACTIONS BETWEEN SPECIES

Organisms interact with each other within ecosystems. These interactions occur between individuals of the same species (intraspecific) and between individuals of different species (interspecific). Some interactions are beneficial to both individuals, such as that between a lorikeet and the flowers that it helps pollinate (Figure 4.2.6). Others may be harmful to one of the individuals, such as when a dingo kills and eats a blue-tongued lizard.

FIGURE 4.2.6 Many flowering plants depend on their ability to attract insects and birds to pollinate flowers and disperse seeds. Rainbow lorikeets feed on the nectar in eucalypt flowers, and at the same time transfer pollen from one flower to another.

Organisms in ecosystems interact in a number of ways. Sometimes two quite different organisms live and function together in a close association, to the benefit of at least one of them. Different species living together in a close partnership is called **symbiosis**. Each species is called a **symbiont**. Mutualism, commensalism and parasitism are all examples of symbiotic relationships.

Interactions between species (interspecific interactions) are usually classified according to how the interaction affects the survival and reproduction of the species involved. These interactions can be classified as:

- benefiting both species (mutualism)
- benign, where one species is not affected by the interaction (commensalism)
- harmful to one species (parasitism, amensalism, predation)
- harmful to both species, although one is usually worse off (competition).

Interspecific interactions can also be classified as feeding and non-feeding interactions. For example, mutualism is a non-feeding interaction, while predation is a feeding interaction.

Interspecific interactions occur between or involve two or more species.

Mutualism

Mutualism is a partnership between two different kinds of organism in which both of them benefit. Some of the most common partners in mutualism are unicellular algae. The bright colours of corals, the green of some hydra and the brilliant blues of the mantles of giant clams are produced by algae living in the animal's tissues (Figure 4.2.7). The algae photosynthesise and produce sugar and oxygen, which are used by the animal partner for its own benefit. In turn, the animal produces carbon dioxide during respiration, which the algae need for photosynthesis. The algae also benefit by having safe shelter within the partner. Some animals may also produce nitrogenous wastes in a form which can be absorbed and used by the algae for the production of nitrogen-containing organic compounds, like proteins.

Emus have a mutualistic relationship with quandong plants (Figure 4.2.8). Emus are often responsible for the dispersal of the quandong's seeds. The emu can digest the soft part of the fruit but not the seed, which it passes out in its droppings. The droppings act like fertiliser and help the seed germinate and grow. The emu and quandong have a mutualistic relationship: the emus benefit from eating the fruit and the quandong benefits by having its seeds dispersed. Consequently, their niches are intertwined.

In some cases, the mutualistic relationship is essential to the survival of the species (obligate mutualism) while in other cases the relationship between the two species is beneficial to both species but is not essential to the survival to either species (facultative mutualism). Either way, as the examples above demonstrate, niches of organisms in mutualistic relationships are interrelated.

FIGURE 4.2.7 Giant clams and unicellular algae have a mutualistic relationship.

FIGURE 4.2.8 The fruits of the quandong are eaten by emus and their seeds are dispersed.

FIGURE 4.2.9 Many owl species nest in tree hollows where it is relatively safe during the day. This is an example of commensalism, because the owl benefits and the tree is not harmed.

FIGURE 4.2.10 Epiphytes form a commensal relationship with the tree they grow on.

Commensalism

Commensalism is an interaction between species in which only one species benefits, but the other species is not harmed. Animals such as birds or possums nesting in a tree hollow is an example of commensalism. In such cases, the bird or possum benefits, but the tree is not harmed (Figure 4.2.9). Niches of organisms in a relationship characterised by commensalism overlap. At other times, such as when birds nest in a tree, one organism becomes the niche of the other.

Trees are also often host to epiphytes: smaller plants such as orchids, ferns, mosses, liverworts and lichens that live on the trunk or in the crown of the tree (Figure 4.2.10). The epiphyte receives sunlight and rainwater. This relationship is usually benign for the tree because it is normally neither helped nor harmed (unless it becomes overloaded with the weight of the epiphytes on its branches). There may also be a fairly insignificant benefit given to the tree from nutrients added to the soil as leaves drop from the epiphyte, so this could be considered a slight mutualistic relationship.

Parasitism

In **parasitism**, one species (the parasite) benefits and the other species (the host) is harmed. **Ectoparasites** such as ticks, lice and mistletoes live on or outside the host (Figure 4.2.11). **Endoparasites** such as parasitic fungi and wood-borers live inside the host (Figure 4.2.12). The host can be considered the niche of the parasitic organism. A parasite obtains its food from the host but does not usually kill it immediately. The type of harm the parasite causes the host varies, but may include:

- shortened lifespan
- impaired functions, such as digestion, photosynthesis or reproduction
- less ability to withstand stresses, such as drought or cold
- greater vulnerability to predators.

FIGURE 4.2.11 Head lice are ectoparasites. They live on the scalps of humans and feed on blood that they suck from the scalp of the human host.

FIGURE 4.2.12 This stick insect has been invaded and is being killed by a parasitic fungus. The fruiting bodies of the fungus are erupting from the insect's body and will release spores that will help the parasite to spread.

Many parasites have more than one host during their life cycle. A host that transfers a parasite to another host is called a **vector**. For example, the malarial mosquito is a vector for the *Plasmodium* parasite. The mosquito transfers the parasite when it bites a person to obtain blood. An infected person is also a vector for *Plasmodium* because they transfer the parasite to the mosquito when the person is bitten.

All organisms have parasites. Every species of plant and animal that has been studied has been found to have at least one parasitic species living in it. Tapeworms are a common parasite in animals (Figure 4.2.13). Tapeworms are ingested and attach to the small intestine where they absorb nutrients and can grow quite large. Although they can cause illness, loss of appetite and anaemia, they sometimes produce no symptoms at all.

FIGURE 4.2.13 Tapeworms are endoparasites that infect animals, including humans.

Amensalism

Amensalism refers to an association between species in which one is inhibited or killed and the other species is unaffected. The niches of organisms in a relationship characterised by amensalism do not overlap.

A simple model of amensalism is the way in which animals can inadvertently damage vegetation around them but are unaffected by the relationship. For example, some waterbirds, such as cormorants, kill vegetation in places where they roost or nest (Figure 4.2.14). Their droppings have a high nitrogen, phosphate and potassium content, which the plants cannot tolerate.

FIGURE 4.2.14 Cormorant droppings can kill vegetation, but this does not harm or benefit the birds.

Allelopathy is a specific type of amensalism where an organism produces and secretes chemicals that affect the growth, reproduction or survival of other organisms. The organism producing the chemical can benefit or be unaffected.

Penicillium are fungi that secrete penicillin, a compound that kills certain bacteria (Figure 4.2.15). This benefits the *Penicillium* by reducing competition for nutrients and space from other microbes. The *Penicillium* may also possibly benefit from a reduction in populations of bacteria that make toxins that harm it. This is an example of amensalism. Penicillin was first developed as a useful antibiotic for combating bacterial infections in the 1940s.

FIGURE 4.2.15 *Penicillium* are fungi that secrete penicillin. A common species is blue mould (*Penicillium roquefortei*), which grows on old bread and is used to make blue cheeses.

Predation

Predation occurs when one animal species (the predator) kills and feeds on another animal (the prey). Predators are carnivores. Some predators hunt for their prey (Figure 4.2.16a), but others catch their prey in traps (Figure 4.2.16b). In both cases, the predator benefits by eating and killing the prey, which is obviously harmed.

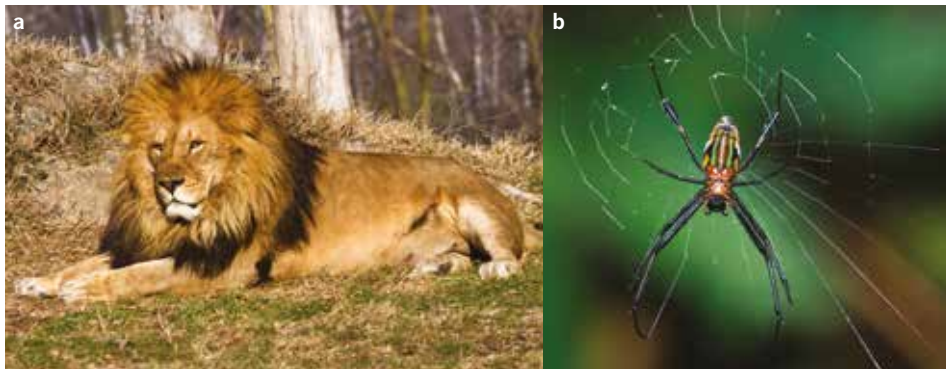


FIGURE 4.2.16 (a) Lions are predators that hunt for their prey. (b) Spiders are predators that create webs to trap passing insects.

Competition

The presence of other organisms may limit the distribution of some species through interspecific competition, resulting in competitive exclusion. Interspecific competition is a struggle between organisms of different species for the same supply of food, water, space, nest sites or any other environmental resource that is in limited supply.

Because they use similar resources, green plants mainly compete with other green plants, herbivores with other herbivores, and predators with other predators.

According to the principle of competitive exclusion, when two species have the same requirements in the same habitat, the species with a more limited tolerance range and less flexible physiological adaptations to interact with the environment will become extinct, thereby eliminating the competition. In other words, the niches of organisms in competition initially overlap. If one organism out-competes the other, then their niches will not overlap.

For example, populations of two species of protists, *Paramecium aurelia* and *Paramecium caudatum*, will grow rapidly in separate but identical cultures, and then both populations will level off (Figure 4.2.17). However, if these species are grown together, the population of *P. caudatum* grows initially, but then its population decreases to extinction. In other words, *P. aurelia* outcompetes *P. caudatum*. *P. aurelia* continues to reproduce to reach a similar population density as in the first experiment in which the two species were separated.

Effectively, this is unlikely to be seen in nature unless there has been a very recent introduction of a new species, such as when an invasive species is introduced from elsewhere.

Interspecific competition occurs between species ('inter' means 'between'). Intraspecific competition occurs within a species ('intra' means 'within').

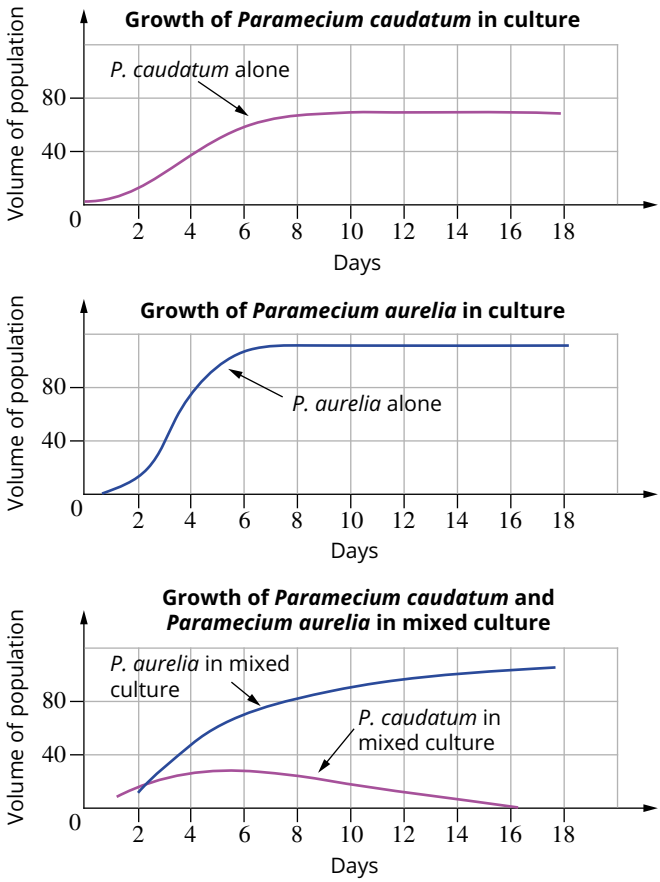


FIGURE 4.2.17 When two species of *Paramecium* are cultured together, the population growth of both species is slowed until eventually *P. aurelia* outcompetes *P. caudatum*.

Competitive exclusion in action: Red fire ant

Several species of scarab beetle are native to Australia. They are generally considered a nuisance as their larvae feed on the roots of pasture plants causing them damage. The beetle's eggs are laid in the soil where the larvae hatch after 2–4 weeks, depending on the species. The larvae then spend several months in the soil before progressing to the adult stage.

While the scarab beetle larvae are a pest to farmers and graziers, they are important to native species as a source of food. Birds such as the Australian magpie and the straw-necked ibis depend on the larvae for food. Many native invertebrates also rely on scarab beetle larvae for food, especially thynnid wasps that parasitise the scarab larvae by laying their eggs into the living grubs. The wasp larvae feed on the scarab beetle grub, eventually killing it. Thynnid wasps play an important role in Queensland ecosystems as they are the sole **pollinators** of several species of orchids. Each orchid is pollinated by a single species of wasp.

The accidental introduction of the red fire ant into Queensland is reducing the abundance of thynnid wasps due to competition for the same food source. The red fire ant is a voracious predator that forms colonies with millions of individuals and many queens. Red fire ants are omnivorous, feeding on plants, birds' eggs and nestlings, invertebrates, small reptiles and mammals, if they can capture them. In the Australian context, it preys on many species of invertebrates, including the scarab beetle.

The aggressive and voracious nature of red fire ants means that few species of ground-dwelling invertebrates are able to remain in the vicinity of a colony due to its competitiveness. In an area where fire ant invasion has occurred, all scarab beetle grubs will be consumed by the ants. This leaves no grubs for the thynnid wasps to parasitise, which leads to the loss of the wasps from the area.

This is an example of competitive exclusion. The red fire ants are not directly killing the wasps, but both the wasps and the ants are in competition for the same resource—the scarab beetle larvae. The red fire ants are the winners in this battle. The likely outcome is that thynnid wasps will become much rarer in Queensland, unless plans for the eradication of red fire ants are successful.

Due to the small number of confirmed infestations, extensive studies on the effects of red fire ants on native populations in Australia have not been undertaken at this stage. However, in the USA, where the introduction of the red fire ant from South America occurred in the 1930s, more detailed studies have been conducted.

A study undertaken in Texas showed that, following the invasion of the red fire ants, native ant species diversity and abundance was significantly reduced. The number of species of native ants dropped by 70%, and even those species that survived in the area were greatly reduced in numbers. The numbers of individual native ants decreased by over 90%. For one sampling study conducted, five pairs of matched sites were compared. The only difference between the matched sites was the presence or absence of red fire ants. Drop pits were dug at each site to capture and identify the species of ants present. The results of the study are shown in Figure 4.2.18. The studies showed that there was little evidence that the red fire ants directly preyed upon the native ants in any significant numbers.

It is possible that competitive exclusion can completely eliminate a species from a particular area, but what is more often observed in natural situations is that the niches of two species overlap. This results in the species that is less able to compete having a smaller realised niche than its fundamental niche.

Animals that transfer pollen are called pollinators. Pollination is the transfer of pollen from an anther (in the male part of a flower) to a stigma (in the female part of a flower).

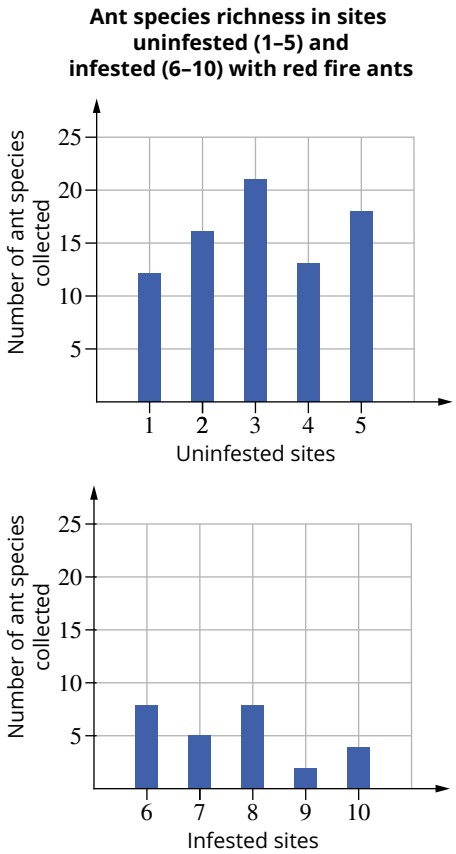


FIGURE 4.2.18 The number of native ant species observed at sites uninfested and infested with red fire ants.

KEYSTONE SPECIES

FIGURE 4.2.19 A keystone is the central stone that supports the arch structure in a stone archway.

Species are interconnected within food webs in ecosystems. Unsurprisingly, when a change occurs to one species, other species and sometimes even the entire food web are affected.

The complexity of a food web gives an ecosystem its stability. In a simple food web, the loss of one species would have a disastrous effect on the other organisms. In a complex food web, the loss of one species has less effect, since alternative food sources are often available.

Some species in an ecosystem can be identified as keystone species. A **keystone species** is a species that plays a critical role in maintaining the structure and functioning of their ecosystem. It is named after the stone at the top of an arch that holds the arch together and gives it strength. Without the keystone, the arch will collapse (Figure 4.2.19). When a keystone species is removed from an ecosystem, the ecosystem becomes much less stable and its structure changes. Keystone species regularly have a much greater impact on their ecosystems than might be expected from their numbers.

The first keystone species

The term keystone species was first applied during a study of food webs in rock pools. The carnivorous sea star, *Pisaster ochraceus* (Figure 4.2.20), was identified as the top predator in the rock pools. As an experiment, all the *Pisaster ochraceus* were removed from one rock pool. A nearby rock pool was left undisturbed as a control. In the test rock pool, the remaining species competed with each other to occupy the extra space and use the additional resources made available. Two types of barnacles and a mussel species began to dominate. They consumed so much of the limpets' algal food source that the limpet population decreased. Within a year, the number of limpet species decreased from 15 to 8. In the control rock pool, there was no change in species number or distribution. As these significant changes resulted from the removal of the sea star, *Pisaster ochraceus* was named a keystone species.

FIGURE 4.2.20 A cluster of sea stars (*Pisaster ochraceus*), the predator species first termed a keystone species.

This experiment showed the huge effect that removing a keystone species from an ecosystem can have.

As keystone species usually have a greater impact on an ecosystem than their numbers would indicate, identification of keystone species is very difficult. It is normally only when they are removed or greatly reduced in numbers in an area, and a major ecological impact is felt, that the importance of a particular keystone species to an ecosystem is noticed.

Example of a keystone species: Great white shark

The numbers of the great white shark have been declining, mostly because they are caught in fishing nets or are hunted (Figure 4.2.21). This has had far-reaching effects on marine ecosystems. The great white shark is a predator at the top of the food chain (apex predator), keeping populations of the fish, seal and sea lion species that they consume in check. The reduction of the fish, seal and sea lions in turn affects the numbers of the animals that those species consume. The great white shark is a keystone species that helps maintain the stability of marine food chains. Until shark numbers declined so significantly, the importance of the great white shark to marine ecosystem stability had not been recognised.

FIGURE 4.2.21 The great white shark is an important predator and a keystone species in marine ecosystems.

The loss of apex predators such as the great white shark often results in a trophic cascade. A **trophic cascade** is a top-down affect where the loss of an apex predator results in a large increase in the number of midlevel predators, also called **mesopredators**. In 2002, a trophic cascade was postulated by scientists to explain the severe reduction in abundance of bivalve molluscs in the waters off the coast of North Carolina, USA. In this area, the apex predators (several species of great sharks) preyed upon the mesopredators (smaller species of sharks and rays). The removal of the apex predators was postulated to have caused a cascade resulting in a complete collapse in the numbers of bivalve molluscs, which were a food of the now-abundant mesopredators.

This resulted in the loss of the scallop (a bivalve) fishing industry, which had operated successfully for more than 100 years. Figure 4.2.22 shows the changes in abundance measured over a period of time of the great sharks (apex predators, top row of graphs), the smaller sharks and rays (mesopredators, middle row of graphs) and the bay scallop (bottom row of graphs) that were the prey of the mesopredators.

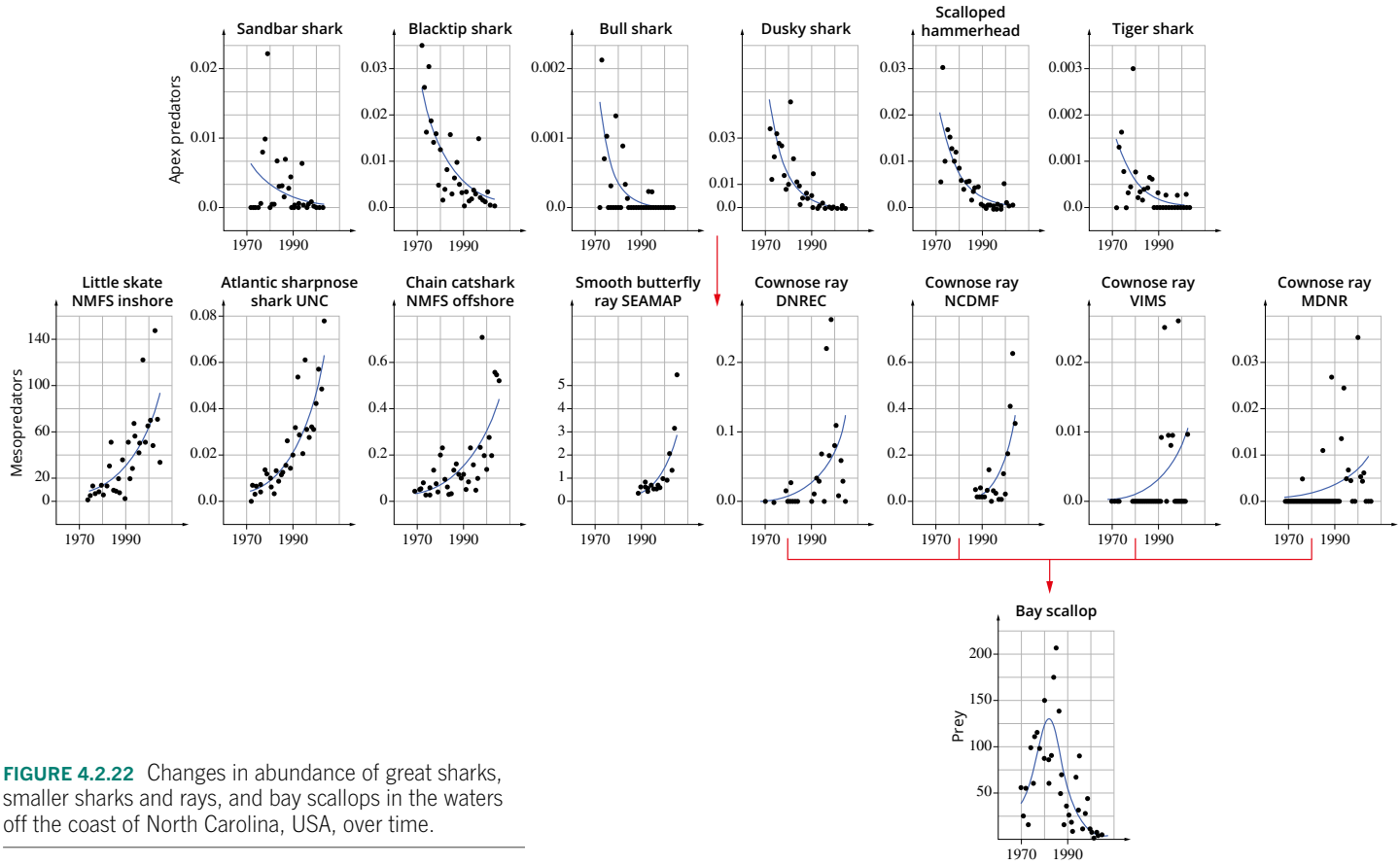


FIGURE 4.2.22 Changes in abundance of great sharks, smaller sharks and rays, and bay scallops in the waters off the coast of North Carolina, USA, over time.

Keystone species and habitat

Some species are keystone species because they affect the habitats of an ecosystem. For example, elephants preserve the grasslands of African savannas by eating young trees (Figure 4.2.23). Without the elephants, the savannas would be invaded by trees and shrubs, eventually becoming forests or shrublands, and many smaller grazing herbivores such as wildebeests and zebras would starve.

Keystone species and human impacts

Human activities can have a detrimental effect on ecosystems, particularly where these activities affect a keystone species. One example is the culling of grey wolves from Yellowstone National Park, USA (Module 2.1). The wolves were originally seen as a pest, but their eradication led to a rapid increase in the elk population, which led to massive overgrazing and the loss of aspen and willow plants. This led to a loss of habitat and food for many smaller species, such as beavers and songbirds, as well as riverbank erosion and increased water sedimentation. In 1995, grey wolves were reintroduced and the ecosystem is slowly recovering.

FIGURE 4.2.23 Elephants are a keystone species in African savannas because they maintain the grassland ecosystem.

CASE STUDY 4.2.1

The cassowary: A keystone species

Identifying a keystone species is challenging. The role that they play in an ecosystem may not be obvious without considerable research but it is possible to establish likely contenders for the role. In one long-term study at Crater Mountain, Papua New Guinea, several species were flagged as being possible keystone species. Three species of bird, Bennett's or dwarf cassowary, Blyth's or Papuan hornbill, and Zoe's or banded imperial pigeon were the most likely candidates.

Crater Mountain is covered in tropical rainforest. The terrain is generally steep and movement of seeds uphill is problematic, so a number of plants rely on animals to disperse their seeds.

In 2012, Ruxton and Schaefer analysed many articles about seed dispersal by animals and concluded that animals with large bodies are important seed-dispersers due to their ability to disperse large-seeded plants. Large-bodied animals can disperse a larger variety of seeds, which aids reproduction for many different species of plants. The contribution of fruit- and seed-eating animals (frugivores) to plant biodiversity and complex ecosystem structures is dependent on a few factors: the ability of the animals to consume various seeds of differing sizes and textures, the physiology of the animal to excrete seeds through their digestive tract mostly intact, and the distance they travel after seed consumption.

In 2009, experiments performed in Kenya by Wahungu and colleagues, summarised in Figure 4.2.24, illustrate the value of seed dispersal to the survival of plant offspring.

In a tropical rainforest, many fruit trees have large seeds and are highly dependent on animals. The fruits of these trees are an essential food source for many of the species of animals living in the Crater Mountain ecosystem. As a result of the importance of the fruit trees as a food source and the need for dispersal of their seeds, researchers postulated that the animals that did this were likely candidates for being keystone species.

During the study, over 400 species of plants were identified as needing seed dispersal. A survey of animals found that there were 31 mammal and 169 bird species present and, of these, at least 29% of the mammals and 47% of the birds consumed fruit. This was an important observation, as most animal dispersal of seeds occurs as a result of seeds passing through the digestive tract of the

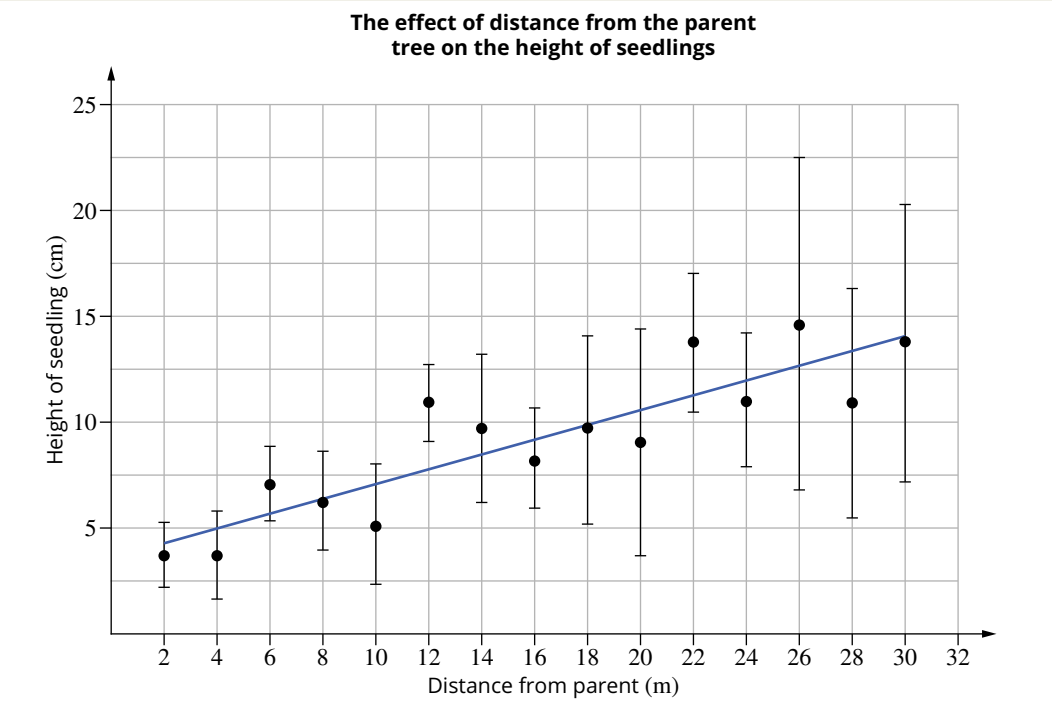


FIGURE 4.2.24 Graph showing the height of tree seedlings plotted against how far they are from the parent tree.

animal and being deposited in the animal's droppings. The investigation concluded that the dwarf cassowary was one of the most important animals involved in seed dispersal.

The value of the cassowary as a seed disperser is related to how it eats the fruit. Many other rainforest animals are frugivorous but most eat only the flesh as they are too small to eat whole fruits. The cassowary, on the other hand, swallows fruit whole, consuming both flesh and seed. Their digestive juices are unable to break down the tough seed coats of most seeds, so the seeds are eliminated with the remainder of other wastes in the cassowary's faeces. Passing through the digestive tract and being excreted with the rest of the cassowary's wastes enhances the chances of germination and growth of the seeds.

Studies have shown that for some species, the germination rate of seeds from cassowary droppings is greater than that of seeds which have not passed through the digestive system of a cassowary. For other animals, there is no difference between the germination rate of eaten and uneaten seeds.

Finally, the seeds spend a considerable amount of time passing through the cassowary's digestive tract. During that time, the bird will travel several kilometres. Cassowaries move seeds a long distance from the parent plant, further enhancing the chances of the survival of an eaten seed and its growth to adulthood.

All of these factors resulted in the conclusion that the dwarf cassowary was a keystone species of Crater Mountain, PNG.

Review

1. Summarise the reasons for deciding that the dwarf cassowary is a keystone species of Crater Mountain in Papua New Guinea.
2. **a** Justify the statement 'Seed dispersal is critical to the long-term survival of most plant species'.
   b State two factors that could account for seed dispersal enhancing survival of individual plants.
3. Explain the importance of the error bars in Figure 4.2.24 and why this is a consideration in ecological studies.

CASE STUDY 4.2.2

Identifying a keystone species of tropical Queensland

Tropical rainforests in north Queensland are known to include at least 1300 species of plants whose seeds are dispersed by animals. The rainforests of north-eastern Queensland are known to contain the habitats of hundreds of frugivorous species. A non-extensive list of the most commonly researched frugivores and seed-dispersers of this area are:

- grey-headed flying fox
- southern cassowary
- imperial pigeons
- musky-rat kangaroo.

Data about frugivores found in north Queensland is summarised in Table 4.2.1.

TABLE 4.2.1 Summary of seed dispersal by animals grouped into mammals and medium-sized birds compared to cassowaries

Organism studied (groups)	Number of seeds the species dispersed	Average (± standard deviation) seed-dispersal distance (m)	Maximum seed dispersal (m)
mammals (general terrestrial)	65	495 ± 221	<1000
medium-sized birds	39	110 ± 109	<550
cassowary	238	337 ± 194	>1000

Many articles have published results showing that mammals and birds are the main frugivores that disperse seeds. Figure 4.2.25 displays the results of one study, showing the number of seeds dispersed by different groups of frugivores and the distribution of seeds.

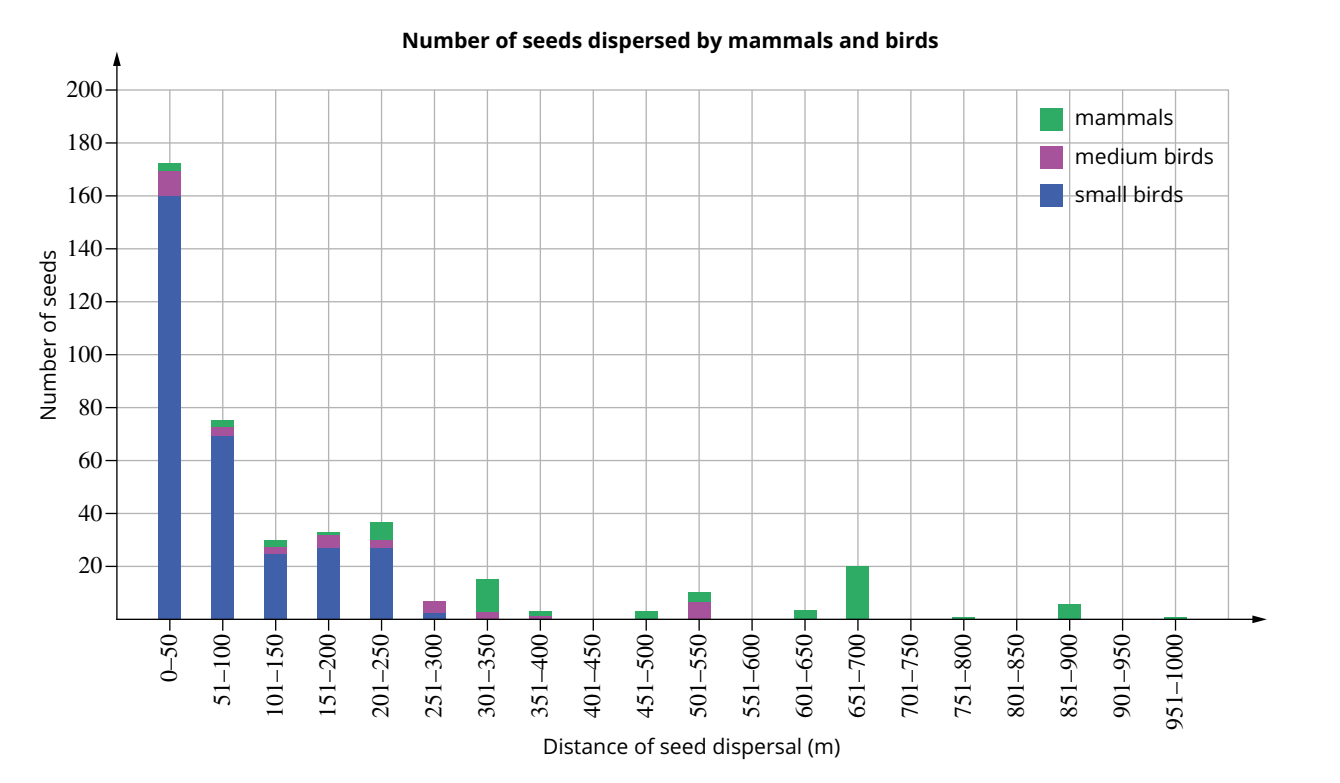


FIGURE 4.2.25 Seed distribution by mammals, small birds and medium-sized birds.

Figure 4.2.26 shows the results of a second study undertaken in the wet tropics of north-eastern Queensland where the number of seeds consumed by various frugivores was investigated. It noted that the cassowary is the only animal that is able to consume seeds of all sizes.

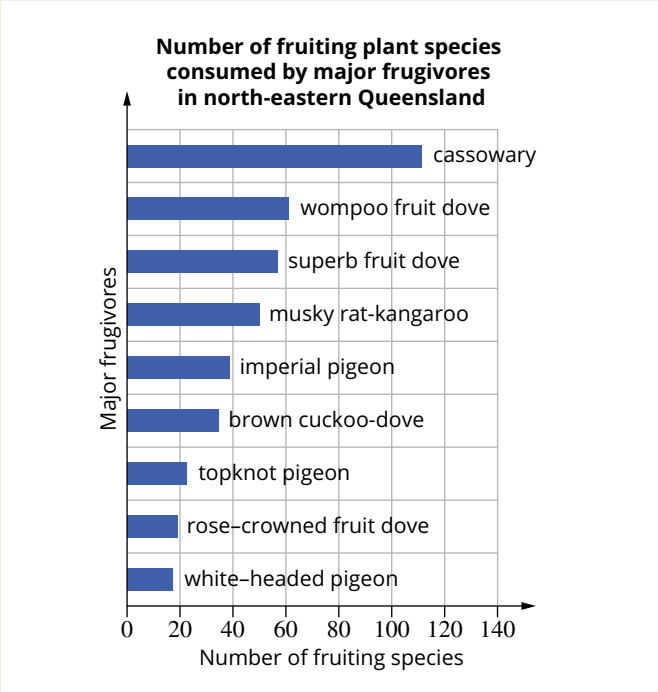


FIGURE 4.2.26 Major fruit-eating species of animals and the variety of plants in their diets.

Review

1 In the north-eastern wet tropics of Queensland, numerous food webs are dependent on the biodiversity of plants for food, nesting, reproduction, camouflage and numerous other requirements, which in turn is dependent on seed distribution. Decide which of the organisms in the data presented is the most likely to be a keystone species. Justify your response.

2 Decide whether medium-sized birds or mammals have the greater potential to sustain a tropical Queensland ecosystem. Argue your decision.

WS 3.2.2

4.2 Review

SUMMARY

- The niche of a species is that part of the environment where the species is most likely to survive, reproduce and persist indefinitely.
- The fundamental niche is the theoretical use of resources for a particular species, while the realised niche represents the actual use of resources.
- Competition is the struggle between organisms for the same environmental resource that is in limited supply. Competition between two species can lead to one species being forced out of its habitat.
- Gause's law of competitive exclusion states that two species requiring the same resources cannot exist in the same niche.
- Niche partitioning is the formation of realised niches that do not overlap, allowing both species to coexist.
- Two organisms can interact with one another in many different ways. The interaction can be harmful or beneficial to either one or both of them.
- Two organisms living together in a close partnership is called symbiosis. Mutualism, commensalism, amensalism and parasitism are examples of symbiotic relationships.
- A keystone species is a species that plays a critical role in maintaining the structure of an ecosystem.
- Keystone species often have a much greater impact on an ecosystem than their numbers would suggest.

KEY QUESTIONS

Retrieval

1 Describe a trophic cascade. Give an example.

Comprehension

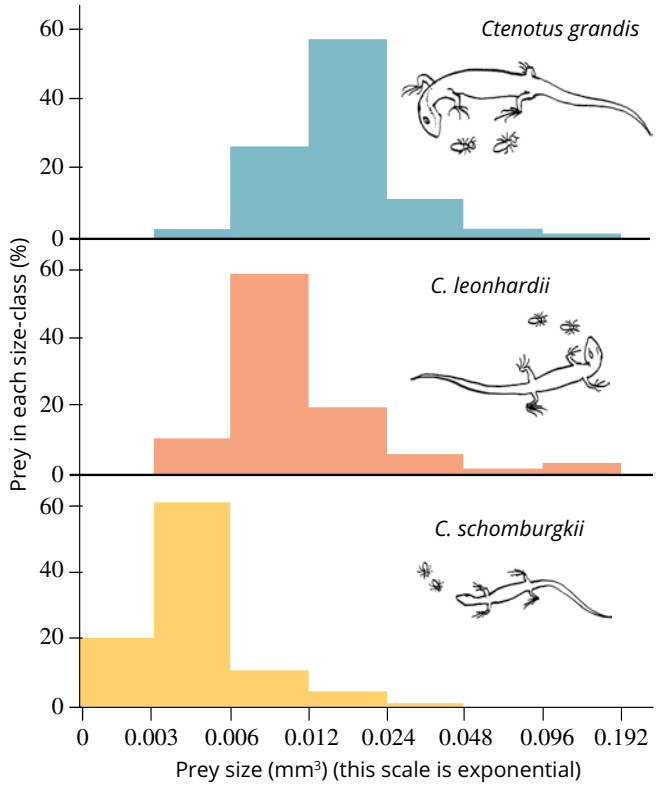
2 The graphs at right show the prey size preferences of three Australian species of lizard.

- **a** Explain how the choice of prey size results in the three species having different niches.
- **b** Identify a limitation in the data and its effect on interpreting the niche of the lizards.
- **c** If the percentage values are indicative of the niches of the three species and there is some overlap, identify for which size of prey there is the greatest amount of competition.

3 A rock pool is a particular habitat. Within this habitat there will be several microhabitats.

- **a** Identify some abiotic factors that could result in different microhabitats in a rock pool.
- **b** It is common for people exploring rock pools to turn over rocks to see what is beneath them. Explain why turning over rocks may lead to the death of the organisms that normally live under the rock.

Prey size preferences of *C. grandis*, *C. leonhardii* and *C. schomburgkii*

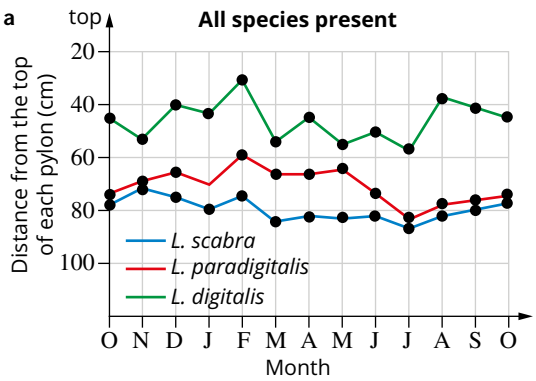


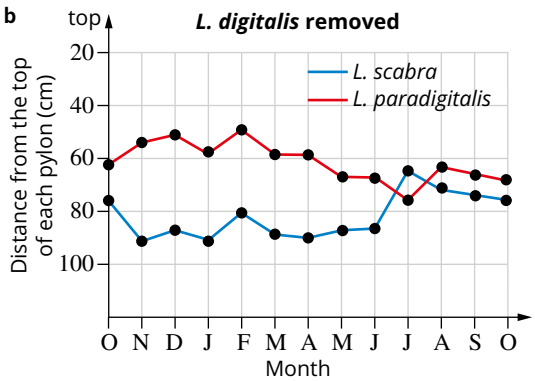
4 Assess and determine the type of biological relationship between the organisms below when farmers allow cattle to graze on agricultural fields and where they use clover to replenish nutrients in a non-crop season.

a clover and root nodule living nitrogen-fixing bacteria
b clover and soil living nitrogen-fixing bacteria
c clover and farmers
d cattle and the ticks that consume cattle blood

Analysis

5 A study was undertaken to examine the interactions of three species of limpets living on pier pylons in California, USA. Three limpet species were examined: *L. digitalis*, *L. paradigitalis* and *L. scabra*. The distance from the top of the pylon was measured for all three species (graph a).





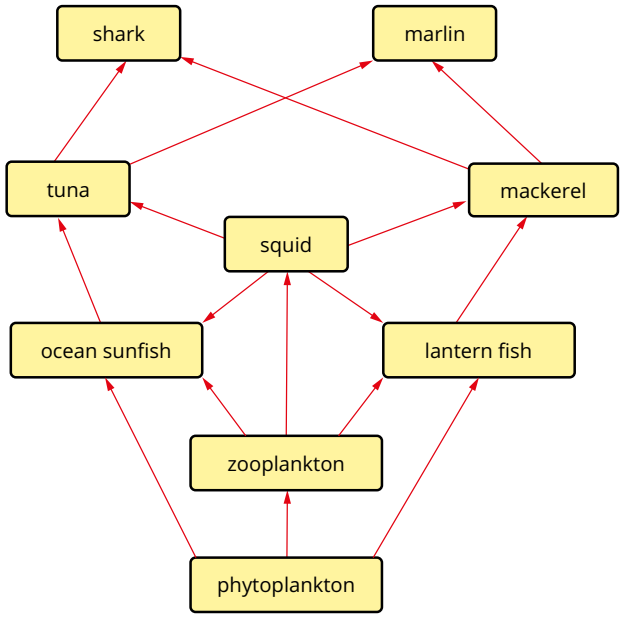
After a period of time, the *L. digitalis* were removed and the resultant effects on the distribution of the other two species was noted (graph b).

a Describe the change(s) that occurred after the *L. digitalis* was removed.
b Deduce the conclusions that can be drawn from these results about the interactions between the three species of limpet.

6 Honeyeaters are nectar-feeding birds found in the Avon wheat belt of Western Australia. They rely on the nectar of flowering plants for food. When consuming the nectar, the birds also collect pollen, which they take to other plants as they feed, leading to pollination of the plants. Most plants in the area flower during the wildflower season (July–November). This is a time of abundance for the honeyeaters. From February to June, there are few plants except the acorn banksia in flower. Explore why the acorn banksia is considered to be a keystone species of the Avon wheat belt.

7 The food web below shows a simple marine ecosystem.

a Identify a potential keystone species in this food web. Explain your choice.
b Predict three possible consequences of the removal from this ecosystem of the keystone species that you identified.



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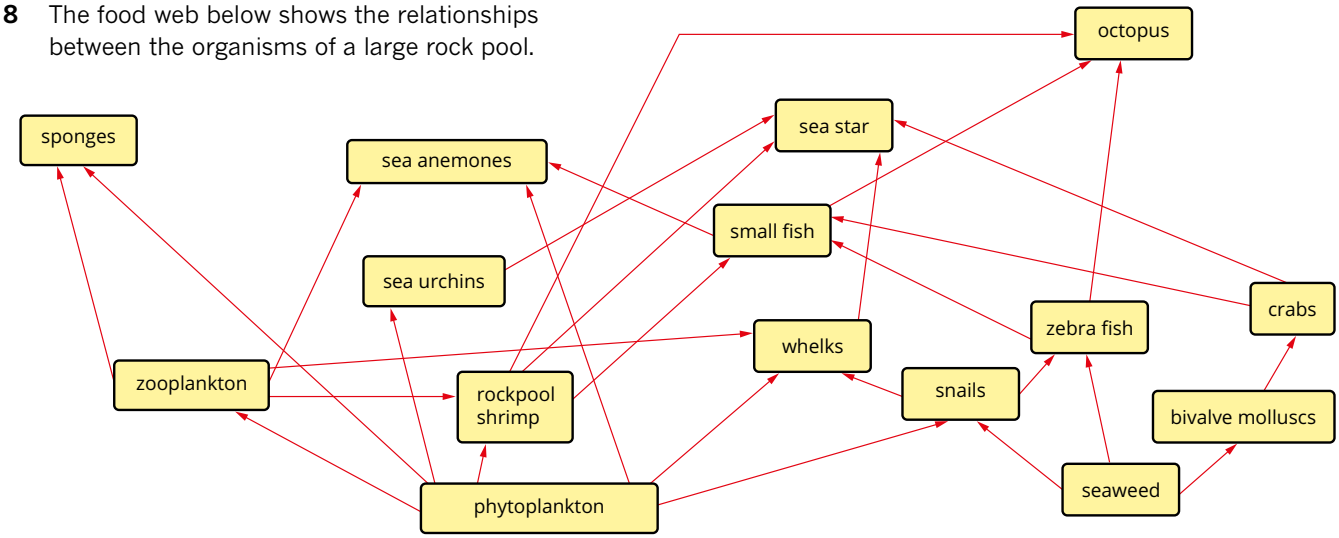
4.2 Review *continued*

8 The food web below shows the relationships between the organisms of a large rock pool.



a Analyse the food web and identify two species that are in competition with each other.

b Infer how the two species that you have identified can both exist in the same ecosystem.

9 The rock pool from question 8 was further studied and the abundance of each organism was determined. The data gathered during the study are shown in Table A. Microscopic organisms were measured as grams per litre of sea water. All other organisms were measured as numbers of individuals.

Table A

Organism	Abundance	Organism	Abundance
phytoplankton	50 g L^{-1} of sea water	rock pool shrimp	27
seaweed	37	octopus	2
zooplankton	20 g L^{-1} of sea water	sea stars	17
sea anemones	5	snails	25
small fish	22	whelks	12
zebra fish	52	bivalves	22
sponges	6	crabs	5
sea urchins	33		

In order to investigate the species interactions in rock pools, a number of artificial rock pools were created that matched the abundance of the species distributions of the natural rock pool described in question 8 and Table A. In a series of experiments, one species was removed from each of the artificial rock pools and the abundance of the remaining species was evaluated after six months.

The results are shown in the tables below.

Experiment 1: Sea anemones removed

Organism	Abundance	Organism	Abundance
phytoplankton	55 g L^{-1} of sea water	rock pool shrimp	27
seaweed	37	octopus	2
zooplankton	30 g L^{-1}L of sea water	sea stars	17
sea anemones	0	snails	25
small fish	22	whelks	11
zebra fish	52	bivalves	22
sponges	14	crabs	5
sea urchins	31		

Experiment 2: Whelks removed

Organism	Abundance	Organism	Abundance
phytoplankton	60 g L^{-1} of sea water	rock pool shrimp	27
seaweed	37	octopus	2
zooplankton	25 g L^{-1} of sea water	sea stars	17
sea anemones	6	snails	25
small fish	22	whelks	0
zebra fish	52	bivalves	22
sponges	6	crabs	5
sea urchins	33		

Experiment 3: Small fish removed

Organism	Abundance	Organism	Abundance
phytoplankton	50 g L^{-1} of sea water	rock pool shrimp	21
seaweed	20	octopus	4
zooplankton	20 g L^{-1} of sea water	sea stars	6
sea anemones	25	snails	12
small fish	0	whelks	2
zebra fish	56	bivalves	18
sponges	12	crabs	5
sea urchins	15		

Experiment 4: Zebra fish removed

Organism	Abundance	Organism	Abundance
phytoplankton	50 g L^{-1} of sea water	rock pool shrimp	26
seaweed	38	octopus	2
zooplankton	20 g L^{-1} of sea water	sea stars	17
sea anemones	5	snails	25
small fish	25	whelks	12
zebra fish	0	bivalves	28
sponges	6	crabs	5
sea urchins	33		

a Analyse the data in the tables and identify which of the four species, if any, could be considered a keystone species. Justify your choice.

b Explore why it is so difficult to identify the keystone species in this ecosystem or any other.

10 Infer why rock pools are useful to investigate theories of ecosystems dynamics.

11 **a** Describe the effect of red fire ants on species richness. Use Figure 4.2.18 (page 165).

b **i** Explain the principle of competitive exclusion.

ii Account for any differences between sites where red fire ants are found and those where they are not.

c Predict the consequences for Queensland ecosystems if red fire ant control measures fail.

4.3 Population dynamics

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand the concept of carrying capacity and the factors that determine it
- calculate the growth rate of a population
- calculate the size of a population using the Lincoln index
- interpret population growth graphs.

Many factors affect population size: availability of space, nutrients and water all have an influence. In order to manage the environment and maintain its health, an understanding of these factors and other influences on population size, growth and distribution is essential. In this module, some of the factors that affect population size and growth will be explored, and one common method of determining population size will be considered.

FACTORS THAT AFFECT POPULATION SIZE

The size of a population is affected by many factors interacting in complex ways. Population sizes vary between species. Some species have very large populations while others have populations that are so small that they are teetering on the edge of extinction (Figure 4.3.1).

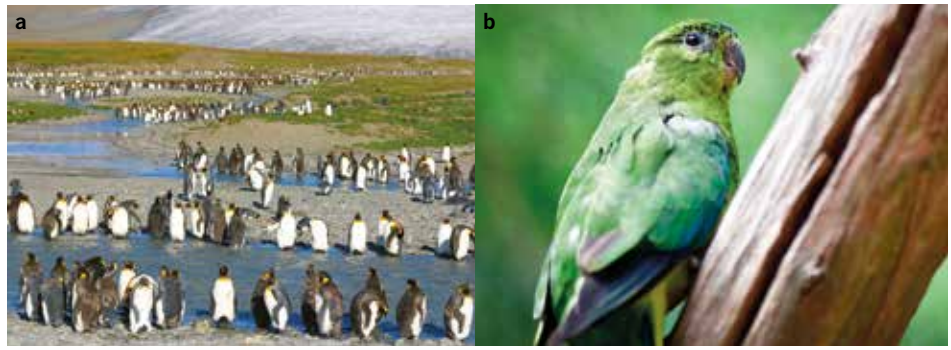


FIGURE 4.3.1 (a) King penguin colonies can consist of hundreds of thousands of penguins. (b) The total population of the critically endangered orange-bellied parrot consists of around 250 individuals (50 in the wild and another 200 spread between seven captive breeding populations).

FIGURE 4.3.2 Individual blades of grass would be difficult to count when measuring population.

Ecologists studying populations usually need to establish their size, but sometimes it is difficult to count individuals. If this is the case, the size of a population can be measured in biomass. For example, counting blades of grass is a tedious process, so a grass population is often measured in kilograms per unit area (Figure 4.3.2). A small area of grass can be cut and weighed, and this value can be used to calculate the total biomass of the population.

The density of a population is also relevant, as the number of individuals is not the only consideration in studying a population. It is also useful to know how closely they are packed together. The density of a population is the number of individuals per unit of area or volume. For example, the number of rabbits in a given area or the number of fish in a particular volume of water.

The area used to measure density might sometimes be represented by a less conventional unit. For example, the population density of plant lice can be expressed as the number of individuals on one leaf (Figure 4.3.3).

There are many reasons for this variation in population size. Human activities, such as land clearing or increasing food supplies, are among them. Also, the effects of one factor can often amplify (increase) the effects of other factors. Any factor that limits the size of a population is called a limiting factor.

The **limiting factor** of a population's size, density or growth is the resource that a population requires but which is present in such small quantities that it negatively impacts the population's growth. For example, food, water, shelter, nutrients and light are essential for a population's growth. If all of these resources except water are available in large quantities, water is the limiting factor and organisms will have to compete for it (Figure 4.3.4). Limiting factors are either density-dependent or density-independent.

FIGURE 4.3.3 The density of plant lice can be measured by counting the number of lice per leaf.

FIGURE 4.3.4 During a drought, water becomes a limiting factor for population growth.

Density-dependent factors

Density-dependent factors are factors that are determined by the size of the population. The effects of these factors increase as the population increases in size. Density-dependent factors influence both birth and death rates in a population. Density-dependent factors include:

- competition for resources, such as food, water, shelter and mates
- predation
- crowding
- parasitism
- infectious disease.

Competition

All organisms have a set of biological requirements for their survival and reproduction. All organisms need resources such as nutrients and water to sustain themselves, shelter for protection and mates for reproduction. If two organisms require the same resource and there is limited access to this resource, there will be competition. As you learnt in Module 4.2, competition can be interspecific (between different species) or intraspecific (between individuals of the same species).

Interspecific competition occurs when different species compete for the same resource. For example, different species might need tree hollows for nesting and shelter. If trees with hollows are blown over or cut down, hollows might become the limiting factor for these species as they will have to compete for available sites (Figure 4.3.5). If one species is more abundant or stronger than the other, the population of the weaker species will decline.

FIGURE 4.3.5 The (a) pink cockatoo needs tree hollows for nest sites and shelter. Other species in the ecosystem, such as parrots, owls, bees and (b) brushtail possums, also compete for tree hollows.

FIGURE 4.3.6 Capuchin monkeys live in a hierarchical group. They are territorial and the strongest individuals obtain much larger quantities of food than the rest. The weakest individuals die.

FIGURE 4.3.7 Piglets compete with each other for access to milk from their mother. Often the smallest piglet in a litter fails to obtain sufficient nutrients and eventually dies.

Intraspecific competition occurs when individuals of the same species compete for a resource. For example, if the food resource of a species suddenly becomes scarce, individuals of the species will have to compete for the remaining food (Figure 4.3.6).

Competition can result in reduced growth, inability to reproduce and death or emigration. Either type of competition is more likely to occur when population densities are high and there is greater demand for resources. However, intraspecific competition is far more likely to occur than interspecific competition, because all individuals in a population have the same basic requirements for survival as they occupy the same niche. Different species generally have different basic requirements for survival, although their niches, and thus some of their needs, might overlap.

Both kinds of competition select for stronger individuals or species. In intraspecific competition, only the strongest individuals will survive (Figure 4.3.7). In interspecific competition, the population of the weaker species may decline (Figure 4.3.8).

FIGURE 4.3.8 Hyenas and vultures compete for the remains of a dead zebra.

Predation

Another way that a species can affect the population of another species is through a feeding relationship such as predation. If the density of the prey species increases, predators will have more access to this source of food and their population will increase. This reduces the population of the prey species, because more predators are eating them. As the number of prey falls, intraspecific competition in the predator population will reduce its population size.

Predator and prey relationships constantly fluctuate in this way. For example, the Canadian lynx preys almost exclusively on the snowshoe hare (Figure 4.3.9). The population of hares varies according to factors such as climate, disease and availability of food. An increase in the hare population leads to an increase in the lynx population. When there are more predators, the hare population may decline because more are killed by lynxes. This in turn may cause the lynx population to decline again. The graph in Figure 4.3.10 shows this repeating cycle.

FIGURE 4.3.9 The (a) Canadian lynx and (b) snowshoe hare have a predator–prey relationship.

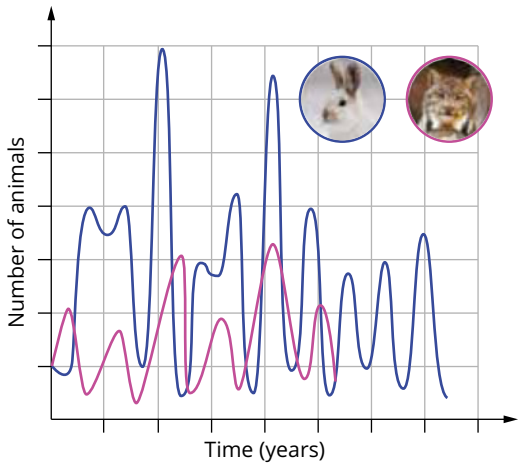


FIGURE 4.3.10 As the number of prey (snowshoe hares, indicated by the blue line) increases, so does the number of predators (Canadian lynxes, indicated by the pink line). Over time, the prey population decreases as a result of predation, which leads to a decrease in the predator population.

Crowding

Crowding affects populations of different species in different ways. For example, when the density of aphids is low, few aphids develop wings, because they do not need to move far to obtain the resources they need. However, as the population density increases, more aphids develop wings (Figure 4.3.11). Winged aphids can only produce about half the usual number of offspring, but they can disperse to areas with more resources and form new populations.

Some animals become stressed when their density is high and may produce fewer offspring, or their immune systems become affected and they will be more prone to disease infection. The density at which negative effects occur is different for each species.

FIGURE 4.3.11 As population density increases, more aphid individuals develop wings.

Parasitism

Parasitism occurs when an organism lives in or on its host for an extended period of time or for its entire life cycle. When a population's density increases, individual organisms have more contact with each other. They are also more likely to be weakened, for example, as a result of limited supply of a resource such as food. This means that parasites are more easily able to invade and spread in a host species of increased density. Most parasites do not kill their host, but they may:

- reduce the host's ability to reproduce
- reduce the host's lifespan
- make the host more prone to disease
- reduce the host's ability to compete
- change the host's behaviour.

Close association between large numbers of humans, such as in primary schools and kindergartens, facilitates the spread of parasites such as head lice between individuals (Figure 4.3.12).

FIGURE 4.3.12 A head louse feeding on human blood. Close association of humans increases the spread of lice.

FIGURE 4.3.13 Some mosquitoes carry a parasite called *Plasmodium*, which causes malaria in humans and other animals. Most human deaths resulting from malaria occur in Africa, because the species of mosquito there has a long lifespan and a tendency to bite humans rather than other animals.

Infectious disease

Diseases may spread faster within a denser population, assuming that conditions are favourable for infection. Disease-causing fungi, bacteria and viruses that can kill individuals may affect the size of the host population. However, organisms in natural environments may be infected with a disease but still be able to survive and reproduce.

A critical factor for any pathogen (disease-causing agent) is the method by which it spreads to other individuals (Figure 4.3.13). The rate at which a pathogen spreads depends on various factors, including environmental conditions like wind, water flow and temperature, and biological factors like the general health and nutritional state of the host. The genetic diversity of the host population can also affect the spread of a pathogen, because a more genetically diverse host population is more likely to have resistant individuals.

Density-independent factors

Density-independent factors affect a population's size regardless of the size or density of the population. They include:

- the conditions in which the species can survive; that is, its daily and seasonal tolerance range for various abiotic factors
- major changes or disturbances to the environment, such as bushfires, droughts or floods.

Tolerance range of a species to abiotic factors

Every species has a particular range of conditions in which it can survive. For each abiotic factor, there is an ideal range that is favourable for the growth, development and survival of the species. Abiotic factors that can affect population size include:

- temperature
- sunlight
- soil or water pH
- salinity
- humidity
- wind strength
- water availability
- pressure (for example, water depth).

Outside its ideal range for a particular factor, an organism will experience stress. Development may be delayed, and survival and reproduction rates and lifespan may be decreased. At a certain point outside its ideal range, the organism will die. For example, a particular species may be able to survive between temperatures of 10°C and –40°C, but its ideal temperature for maximum survival and reproduction may be 25–30°C. At temperatures below 10°C or above 40°C, death is likely. When graphed, an organism's tolerance to an abiotic factor is often a bell-shaped curve (Figure 4.3.14).

Outside the optimum range for an abiotic factor, the productivity and abundance of a species is reduced. One abiotic factor affecting the productivity of agricultural crops is soil pH (Figure 4.3.15).

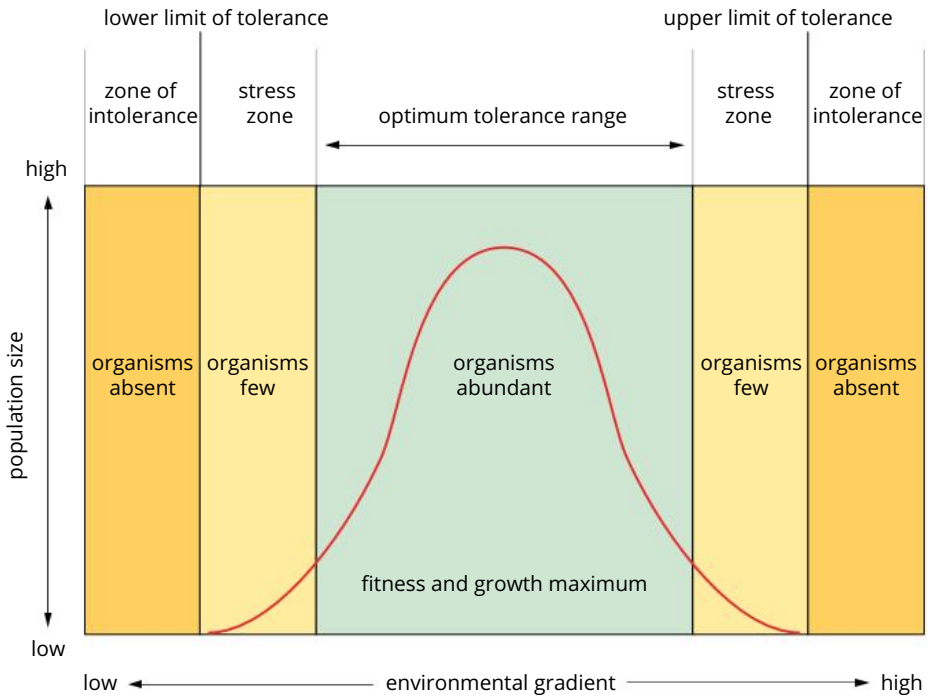


FIGURE 4.3.14 An organism's tolerance to an abiotic factor is often a bell-shaped curve (shown here with a red line). As the environmental gradient increases and decreases, fewer individuals are seen.

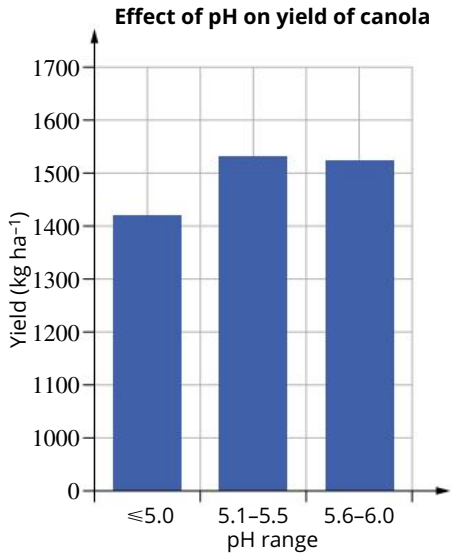


FIGURE 4.3.15 Outside the optimal pH, the productivity of canola crops is reduced.

Salinity of the soil also influences the survival of canola seeds and seedlings, and thus influences overall productivity. Soil salinity is measured in deciSiemens per metre ($dS\,m^{-1}$), which is a measure of how well the soil conducts electricity. As Figure 4.3.16 shows, canola can tolerate low levels of salinity. However, at levels of 12.4 $dS\,m^{-1}$, seed and seedling survival are severely impacted. Very few plants can thrive in highly saline soils (Figure 4.3.17).

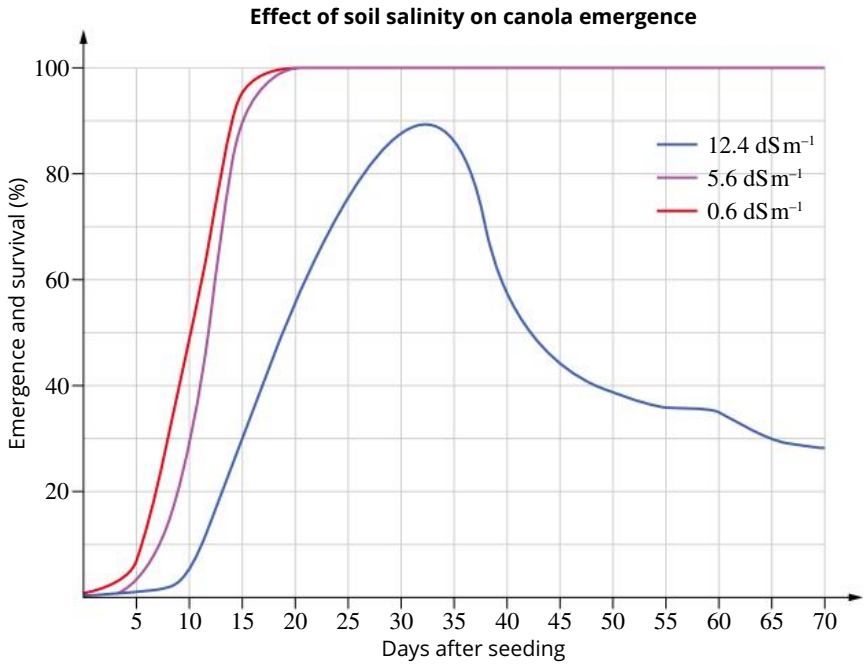


FIGURE 4.3.16 The effect of soil salinity on the survival of canola seeds and seedlings at three different soil salt concentrations.

FIGURE 4.3.17 Soils high in salt are not suitable for the growth of many plants. The plants that do grow are low in productivity.

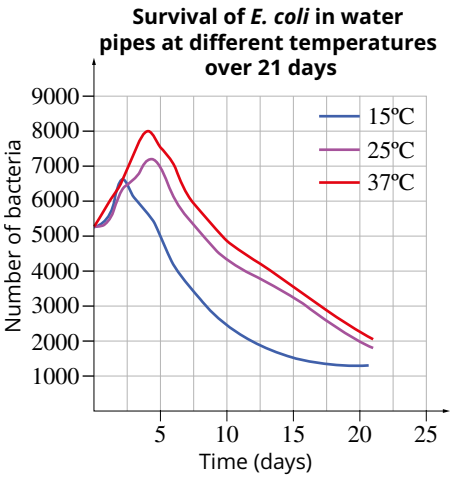


FIGURE 4.3.18 The survival and reproduction of *E.coli* at three temperatures: 15°C, 25°C and 37°C.

Organisms are also affected by temperature. Each organism has an optimal temperature range. The range varies between species. For example, *Escherichia coli* are bacteria that infect humans. They have an optimal temperature which is close to that of human body temperature (37°C). When grown outside the optimum temperature, growth rates and survival are reduced. This can be seen in the results of an experiment showing how well *E. coli* survive in water pipes (Figure 4.3.18).

Major changes to an environment

Other types of density-independent factors affecting population size are those related to sudden or major changes to an environment. These can occur in a number of different ways, some natural, some caused by human activity, and some a combination of both, including:

- natural disasters, such as flood, bushfire (Figure 4.3.19a), drought, volcanic eruptions, tsunamis, earthquakes and cyclones
- human-made changes, such as construction or pollution
- combination changes, such as global climate change.

Natural disasters and human-made changes can have wide-ranging effects on plant and animal species. The destruction of habitat (Figure 4.3.19b) can displace many organisms, and some major changes to the environment can kill organisms (Figure 4.3.19c).

WS 3.2.3

FIGURE 4.3.19 (a) Bushfires affect many animal and plant populations. (b) Clearing rainforests for oil palm plantations in Malaysia destroys habitat for many species, including elephants, tigers and orangutans. (c) Pollution of waterways reduces oxygen levels and causes the death of fish and other aquatic organisms.

Calculating the size of a population

Ecologists Frederick Charles Lincoln and C.G. Johannes Petersen devised a method known as the Lincoln–Petersen index, or the **Lincoln index**, that can be used to determine the size of a population of animals. Estimating population sizes of animals that are mobile and tend to hide from humans provides challenges that plants don't present. Sampling methods like quadrats and transects, which are useful for determining the abundance and distribution of plants, are not viable when the target organism can move. You learnt about sampling methods in Module 2.3.

The Lincoln index uses a method known as capture, mark, release and recapture to estimate the size of an animal population. Normally, several sampling sites would be randomly chosen and members of the target species would be captured at each of the sites. Each captured individual would be marked in some way that did not reduce their chances of survival and reproduction, and then released. Some time later, the sampling would be repeated and the number of tagged and untagged individuals noted. A calculation could then be performed to provide an estimate of the size of the population.

The formula is given as:

$$N = \frac{M \times n}{m}$$

where

N is the size of the target population

M is the number of individuals caught, tagged and released in the first sampling

n is the number of individuals caught in the second sampling

m is the number of tagged individuals caught in the second sampling.

For example, if the number of individuals caught in the first sample was 150 and in the second sample was 120, with 25 of those having been previously tagged, the estimated population would be:

$M = 150$

$n = 120$

$m = 25$

$$N = \frac{150 \times 120}{25}$$

$$= 720$$

As with all sampling methods, the Lincoln index only gives an estimate of the population of any species. If a survey of all of the species in an area is being undertaken, the researchers should keep in mind that rare species can be missed.

POPULATION GROWTH

In Module 4.1, you learnt that a population is a group of organisms of the same species living in a defined geographical area. In theory, populations should continually increase in size as a species produces more individuals. However, this is rarely the case in any ecosystem. Instead, population size and density are determined by a variety of factors which interplay to influence:

- **natality** (births or germination)
- **mortality** (deaths)
- **immigration** (organisms moving in from outside the population)
- **emigration** (organisms moving out of the population).

Birth and immigration bring new population members and increase the population size. Death and emigration decrease the population size. Immigration and emigration are collectively known as **migration**. These four processes determine the change in a population, and when calculated for a specified time it determines the rate of change in a population (Figure 4.3.20).

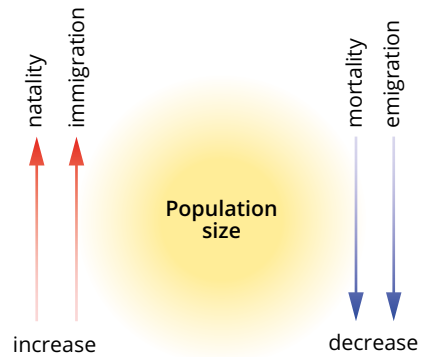


FIGURE 4.3.20 The size of a population depends on natality rate, mortality rate and migration.

For example, a population of kangaroos was studied for five years. During that time there were 200 births and 100 deaths. During the same five years, 50 kangaroos immigrated into the population and 30 emigrated out of it. The total change in population size for the period of five years was:

$(200 + 50) - (100 + 30) = 120$

The population increased by 120 individuals. To calculate the annual rate of change, divide by time, in this case, 5 years. The rate of increase was:

$$\frac{120}{5} = 24 \text{ per year}$$

Where populations decrease in size over the study period, the rate will be a negative value.

Most populations in undisturbed ecosystems have reached a state of equilibrium, where the numbers of individuals may fluctuate in the short term but are fairly stable in the long term. Disturbances to ecosystems can result in large and long-term changes to the number of individuals. Such disturbances can be natural, such as floods and fires, or caused by humans, such as deforestation or nutrient loading.

As long as the birth rate is higher than the death rate, a population will grow. Populations that have invaded new territory, are recovering from disturbances or have life cycles with periodic large fluctuations in numbers will have periods when birth rates dramatically exceed death rates but, even in these populations, a point is eventually reached where some necessary resource is at its maximum availability. At this point, population growth will cease. This scarcity of this resource is a limiting factor. All populations eventually experience one or more limiting factors.

Population growth is described by the equation:

population growth = (births + immigration) – (deaths + emigration)

If population growth is positive, the population is growing. If population growth is negative, the population is declining. If population growth is zero, the population is stable and is said to be in equilibrium.

Exponential population growth

If the birth rate of a population remains consistently higher than the death rate, the population may grow exponentially. Species that tend to experience exponential population growth are those that have a short generation time and give rise to large numbers of offspring. Examples are bacteria, many weed species and some types of insects. In most instances, exponential population growth in these species occurs only for relatively short periods of time.

Ecologists use mathematical formulas to model the theoretical growth of a population over time. The graph in Figure 4.3.21 shows a theoretical growth curve for a population in an ideal environment. The graph assumes that the number of immigrants equals the number of emigrants over time. Change in this population is therefore a function of births and deaths only. This type of growth is known as **exponential growth**. The J-shaped curve of the graph is characteristic of exponential population growth.

Example of exponential growth

Year	0	1	2	3	4	5	6	7	8	9	10
Size of population	100	200	400	800	1600	3200	6400	12 800	25 600	51 200	102 400

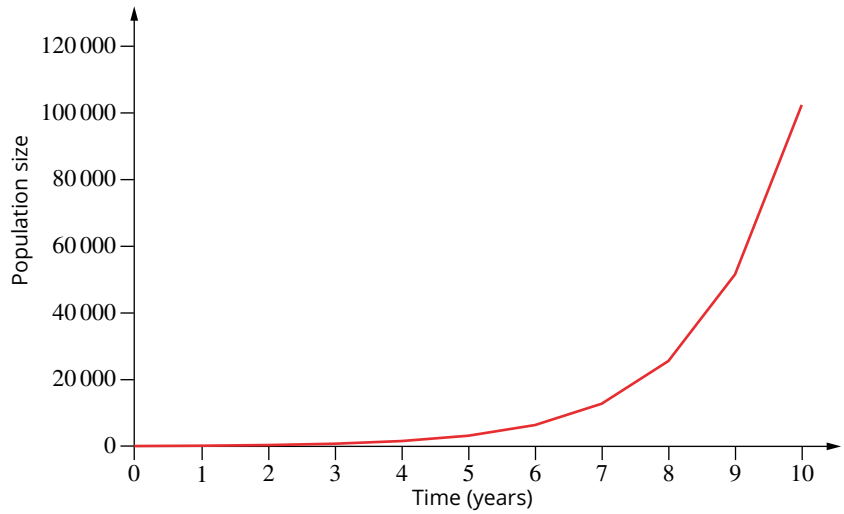


FIGURE 4.3.21 The size of a population over time, showing exponential population growth.

Exponential growth is normal for some plants and animals when environmental conditions are favourable and resources are abundant (plentiful). Because these conditions generally last only a short time, exponential growth is usually short-lived. But if favourable conditions continue then a **population explosion** may occur.

Salvinia fern is a free-floating aquatic weed that often has population explosions (Figure 4.3.22). It can survive for up to 20 months in dry conditions, but under favourable environmental conditions, such as high nutrient levels, it can double its population every two to five days. If these conditions continue, Salvinia fern will form a dense mat on top of a waterway, preventing other aquatic plant life from receiving sunlight. Due to its growth rate and damage to aquatic habitats, this introduced species is a declared prohibited weed throughout Australia.

Organisms that reproduce during a particular period of the year often have massive increases in their population during this time, and decreases in population throughout the rest of the year. One example is the sea turtle. Sea turtles come ashore once a year and bury large numbers of eggs in the sand. When the eggs hatch, the turtle population is very large; however, very few baby turtles make it safely to the water or survive predation in the ocean.

Other species reproduce even less frequently. Periodical cicadas live underground for up to 17 years before emerging to reproduce, when the females lay hundreds of eggs in three or four weeks. The population growth at this time is tremendous, but only occurs for a short time (Figure 4.3.23). Exponential growth may also occur when an organism invades a new environment; for example, when cane toads were introduced into Australia.

FIGURE 4.3.22 Salvinia fern is one of the world's most troublesome aquatic weeds.

FIGURE 4.3.23 Periodical cicadas live most of their long life underground before emerging to reproduce.

Logistic population growth

In the absence of a limiting factor, the population growth of a species will be continuously exponential. However, in the real world, population growth is affected by density-dependent factors such as competition for resources.

When a population reaches **equilibrium** and becomes relatively constant, with the number of births and deaths in the population cancelling each other out, the species has reached the maximum population size that the ecosystem can sustain indefinitely. This is called the ecosystem's **carrying capacity** for that species. The carrying capacity is the size of the population that can be supported indefinitely on the available resources and services of that ecosystem.

The S-shaped curve of the graph in Figure 4.3.24 shows the initial exponential growth of a population, which then flattens out as it begins to be affected by density-dependent factors. The population growth rate will decline until birth and death balance each other and the population is limited to the carrying capacity of the environment. This pattern of growth is known as **logistic growth**.

Equilibrium is a balance between opposing factors that keeps a parameter (such as population size) stable. For population size, these include the balance of births and deaths, immigration and emigration.

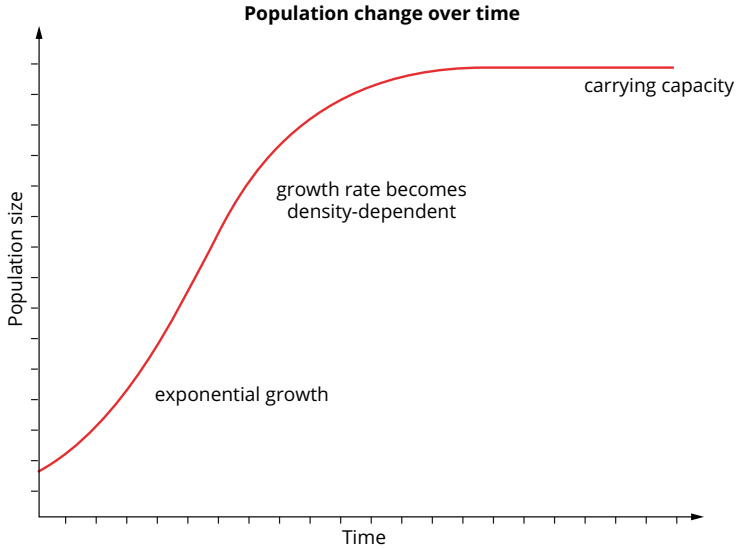


FIGURE 4.3.24 As a population increases, density-dependent factors may become important.

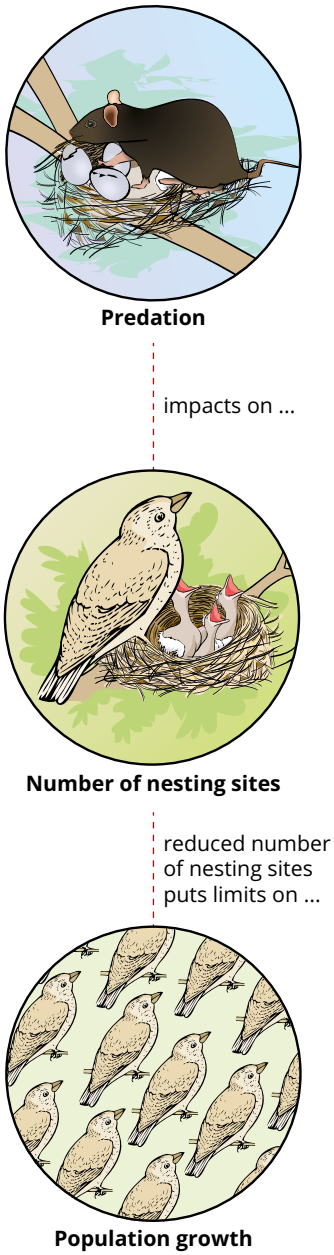


FIGURE 4.3.25 Birds that cannot find a nesting site or sufficient food to raise their young cannot reproduce, which affects population growth. Predation also limits the growth of a prey population.

TABLE 4.3.1 Abiotic and biotic factors that can affect carrying capacity

Abiotic	Biotic
soil	fluctuation of prey species
water	fluctuation of predator species
space	fluctuation of species that compete for resources
shelter	

Consider the following example. A new volcanic island emerged in the middle of an ocean and became populated with plants and insects. A few birds of one species happened to be carried to the island by strong winds. They raised their young and fed on the seeds, insects and fruit available. As they were the only bird species, they had no competition for space or food and no natural enemies, so the population grew rapidly. Eventually there was no space left for new nests. Seeds, fruit and insects became scarcer, and then rats arrived on the island by ship and began eating the birds' eggs. The bird population then levelled out (Figure 4.3.25).

The graph in Figure 4.3.26 shows the changes in the size of the bird species population from the time of their arrival until the arrival of the rats. This pattern of growth is observed in every population in a natural environment. After the arrival of the rats, a predator–prey relationship that caused the population to fluctuate was observed.

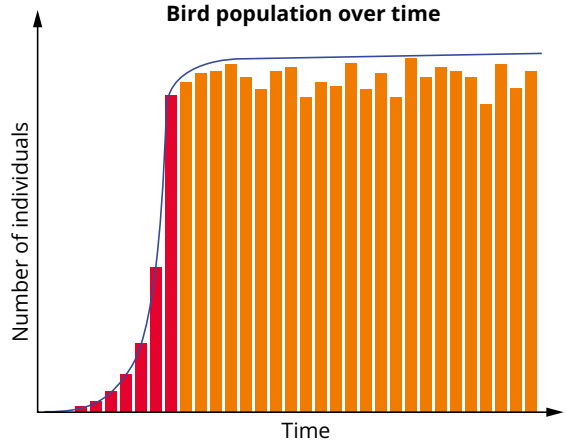


FIGURE 4.3.26 Initially, the bird population grew increasingly quickly (red columns). When the population size reached the point where nesting areas and food were scarce, the population stabilised (first three orange columns). The arrival of the rats caused the population size to fluctuate (remaining orange columns).

In the laboratory, it is possible to create a situation where a population remains absolutely constant over long periods of time. However, in natural ecosystems, this is unlikely. The complex nature of predator–prey relationships means that the actual population size will fluctuate either side of the ideal population size or carrying capacity of the environment.

The dynamic nature of carrying capacity

The carrying capacity of an environment is dynamic, meaning it varies over time. All biotic and abiotic factors are variable, changing in response to both density-dependent and density-independent factors. For example, consider food availability for a herbivore. Food availability can be a limiting factor for population size, but the amount of plant food available is itself dependent on a range of factors that constantly change, including density-independent factors such as weather conditions and density-dependent factors such as plant trampling. Thus, factors that can affect carrying capacity include:

- weather and climate changes
- major changes to an environment
- fluctuation in populations of food species or competitors.

The factors that affect carrying capacity can be abiotic or biotic (Table 4.3.1). Water availability is an example of an abiotic factor that can affect carrying capacity. For example, during a drought, water availability might become the limiting factor for a population of kangaroos. The number of individuals that the environment can support will be reduced.

During summer, it is not unusual to hear that the rivers and waterways of inland Australia are experiencing **algal blooms**. Algae require nutrients, such as phosphates

and nitrates, for growth. When these nutrients are low, algal reproduction is generally slow. Rivers and other waterways acquire these nutrients when nutrient-carrying run-off or sediments are washed into them. Throughout most of the year, the volume of water in the lakes and rivers is high and water moves quickly, flushing nutrient-carrying sediments down rivers to the sea, so concentrations of phosphates and nitrates remain relatively low. However, in periods of low rainfall such as summer or during droughts, the water moves slowly and flow may even cease altogether. Water volumes in lakes and streams are much decreased, due to low rainfall combined with evaporation, so the concentration of dissolved nutrients increases. Reduced flow rates also result in the deposit of nutrient-carrying sediments.

The increase in concentration of nutrients increases the carrying capacity of the waterway for algae. Under these circumstances, the algae can reproduce rapidly, resulting in an algal bloom. In some areas, fertilisation and irrigation of crops (such as bananas and sugarcane), along with increased soil erosion associated with planting and harvesting crops, can exacerbate this process. A 1997 study undertaken in Queensland showed that total phosphorus run-off into surrounding waterways from banana and sugarcane fields (7 kg^{-1} y^{-1}) greatly exceeded that from either pasture or rainforest (2 kg^{-1} y^{-1}), supporting the contention that farming can increase the incidence of algal blooms. Ultimately, the influence of farming on algal blooms due to nutrient loading of waterways will vary from area to area, depending on the types of crops and the farming practices in use.

FIGURE 4.3.27 The algal bloom outbreak in this pond causes a dense green scum on the surface of the water.

Changes in the biotic environment can also cause alterations to the abiotic environment and subsequently change the carrying capacity for certain species. For example, as the algae increase in numbers, they form a scum on the surface of the waterway (Figure 4.3.27). This dense scum blocks the light from plants living below the surface, resulting in their death. The carrying capacity of the waterway for bottom-dwelling plants decreases as light becomes a limiting factor.

The activity of decomposers increases as plants die, as larger amounts of detritus build up on the floor of the waterway. Decomposition requires oxygen, so the water towards the bottom of the waterway becomes oxygen-depleted. In this way, living organisms, the algae, are causing a change in an abiotic factor, oxygen availability. Oxygen availability is a limiting factor for many aquatic species, including fish, tadpoles and insect larvae. As the oxygen is used in decomposition, it is no longer available to these animals. Their numbers will be reduced as they compete for this scarce resource. The organisms that compete best and obtain most access to oxygen will be reduced the least. Some species may become locally extinct. This process is summarised in Figure 4.3.28.

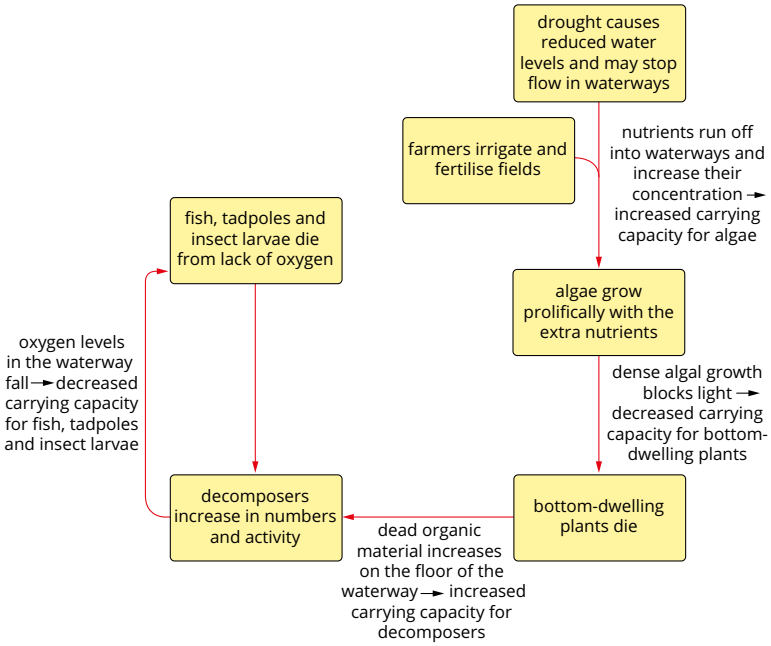


FIGURE 4.3.28 Carrying capacity can alter as a result of changes in either biotic or abiotic factors. Often one change can trigger a series of changes that affect a variety of organisms.

When the rains arrive and the nutrients in the river return to normal levels, the river will no longer be able to support such large number of algae. There will be insufficient nitrates and phosphates to support such a large population. The individuals will compete for what has become a scarce resource, a limiting factor, and most will die. This is called a **population crash** and it occurs when a population exceeds the carrying capacity of its environment.

Population crashes occur because a previously readily available resource suddenly becomes scarce. This may be due to some random change in the environment, such as a fire or flood, or it may be due to human activities, such as over-fishing reducing the availability of a prey species.

Ultimately, the carrying capacity of any environment is tied to the availability of the scarcest resource. If its availability alters, or if another previously readily available resource becomes scarce, this alters the carrying capacity of the environment. If the availability of formerly scarce resources increases, so does the carrying capacity.

WS 3.2.5 | PA 3.2.1 | PA 3.2.2 | SAT IA1

4.3 Review

SUMMARY

- A limiting factor on population size is any factor that stops an increase in the size of that population.
- Food availability affects the movements, distribution and abundance of organisms.
- Population growth can depend on the number of individuals in a given area (density-dependent) or other factors in the environment (density-independent).
- Density-dependent factors include competition, predation, crowding, parasitism and infectious disease.
- Density-independent factors include tolerance range and major changes or disturbances to the environment.
- The characteristics of organisms, including their behaviour and tolerance limits, affect the way that they can respond to their environment and limit where they can grow and their population size.
- Herbivores and carnivores can affect the distribution and abundance of the species on which they feed.
- The distribution and abundance of some organisms are controlled primarily by their interactions with other species.
- The distribution and abundance of some organisms are controlled primarily by the limitations of the physical environment.
- Population size can be calculated using the Lincoln index, which is a formula that uses the capture and recapture method.
- The size of a population is affected by four processes: natality (birth), mortality (death), immigration and emigration.
- In the absence of controlling factors, population growth is exponential. When graphed, it shows a J-shaped curve.
- As a population increases in size, environmental resources become more limiting. Under these circumstances, population growth is density-dependent and slows down.
- Real populations eventually experience limiting factors, population growth stops and the size of the population becomes stable. This is logistic growth.
- The size of a population will reach a maximum at the carrying capacity of the environment. Because of other factors, such as environmental change or catastrophe, the carrying capacity is often not reached.
- Species that have population explosions have a high reproductive ability and often a high dispersal ability. A change in the physical environment or lack of predator control can trigger rapid population growth.
- If the size of the population exceeds the carrying capacity, or if the carrying capacity of an environment reduces suddenly, the population can crash.

KEY QUESTIONS

Retrieval

1 Define:
   a logistic growth
   b intraspecific competition
   c carrying capacity

2 Describe the effect of emigration and immigration on population size.

3 Define limiting factor.

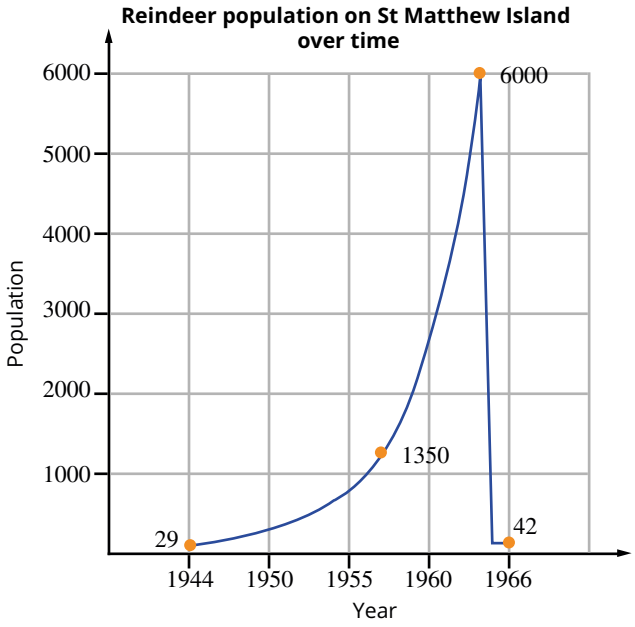
4 **a** Recall what is meant by a density-dependent factor.
   b State two examples of density-dependent factors.
   c State two examples of density-independent factors.

Comprehension

5 Predator–prey population graphs often show peaks and troughs. Explain why the carrying capacity of a predator population fluctuates.

Analysis

6 During World War II, the US Coast Guard introduced 29 reindeer to St Matthew Island in the Bering Sea. The intention was to farm the reindeer for food, but this did not eventuate and the island was abandoned at the end of the war. The reindeer were counted periodically. By 1963, the animals were showing signs of stress, such as poor coat condition, and many were underweight. The lichen on the island had almost disappeared and the reindeer were feeding on less nutritious plants such as sedges. When observers returned in 1966, the island was scattered with reindeer skeletons. By the 1980s, there were no reindeer left on the island. Refer to the graph below.



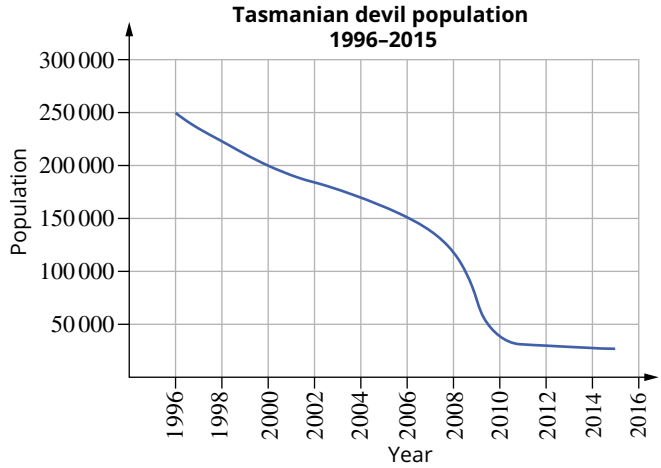
a Identify the growth pattern shown by the reindeer between 1944 and 1963.

b State the reindeer population in 1960.

c Determine the change in reindeer population between 1963 and 1966 and infer a plausible reason.

d Hypothesise why reindeer populations do not suffer similar events in the mountainous regions of Europe and North America, where they are a native species.

7 Early European settlers found Tasmanian devils unnerving and feared that they would attack their livestock. They hunted them extensively and Tasmanian devil numbers declined significantly. In 1941, the Tasmanian devil was declared a protected species and their numbers bounced back to a healthy population size. The security of the population seemed assured until 1996, when the first instance of devil facial tumour disease was observed in Tasmanian devil populations. The population of Tasmanian devils has been estimated since then. The graph below shows one set of estimated population values for Tasmanian devil populations.



Use the data in the graph, along with your knowledge of population dynamics, to answer the following questions.

a Explain why, when considering changes in population size in Tasmanian devils, both immigration and emigration can be discounted.

b Estimate the approximate Tasmanian devil population in 1994. Justify your suggestion.

c Calculate the mean annual rate of decline of the Tasmanian devil population between 1996 and 2002.

d Female Tasmanian devils produce four offspring per year and are generally successful in raising all four until weaning. The population of Tasmanian devils is approximately evenly distributed between males and females, with about half being sexually mature adults.

i State how many adult females were in the Tasmanian devil population in 1996.

ii Calculate the number of deaths in the Tasmanian devil population in the 1996–97 year if every adult female produces four offspring. Show all working out.

8 In one study using capture, release, recapture to estimate population size, 625 individuals were captured and marked. One month later, the traps were reset and 589 individuals were captured. Of these, 126 were marked. Use the Lincoln index to calculate the size of the population. Show your working.

continued over page

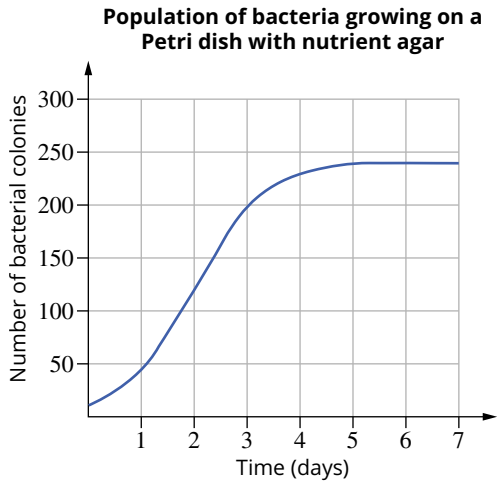
4.3 Review *continued*

9 The following year, a new study was done of the population in question 8 and the following data were acquired.
initial capture numbers = 793
recapture = 797
marked = 145

a Calculate by how much the population has changed.

b Calculate the percentage change in population. Give your answer to one decimal place.

10 Bacteria were grown on an agar plate in the laboratory for seven days at a temperature of 18°C. The growth was monitored throughout that time and the number of colonies was noted each day. The results of the study are shown on the graph below.

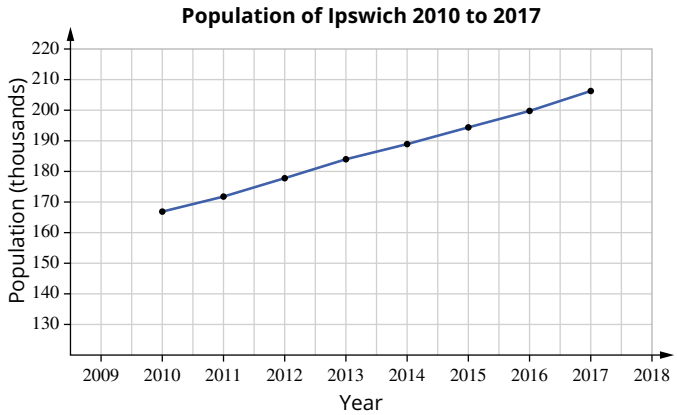


a Identify the kind of growth shown by the bacteria.

b Explain why the population does not keep expanding after day 5.

c Predict how the growth of the bacterial population would change if the same experiment were to be performed but the temperature was raised to 36°C. Explain your reasoning.

11 The population of Ipswich showed a steady increase in size over the period 2010 to 2017.



a Calculate the average rate of change in the population over this period, rounded to the nearest whole number.

b Express the average annual increase as a percentage.

4.4 Change in ecosystems

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand that communities change over time
- know the difference between pioneer communities and climax communities
- know the characteristics that fit an organism to be a member of a climax or pioneer community
- understand how the fossil record is used to show how the environment has changed through geological periods of time
- understand the processes that shape the communities that form on new or disturbed lands and how that affects the organisms living there
- understand how humans and their activities influence the living and non-living environment.

Ecosystems and the communities they support are not static. They change through time. Change happens as a result of seasonal factors, such as rainfall and temperature. It also occurs as a result of sudden random events, such as bushfires, cyclones and floods, or as a result of human activities, such as the clearing of land for agriculture. Following disturbance, ecosystems go through stages of recovery. Ecosystems also develop following the creation of completely new environments, such as after volcanic eruptions.

SUCCESSION

When ecosystems are observed over long periods of time, the relative abundances of different species may be seen to change. Some species will out-compete and replace others. Parts of the environment, such as the soil, may also be observed to change. The ecosystem may eventually look completely different. This process of change takes place in an orderly manner, called **succession**.

One well-documented case of succession occurred in the USA when unproductive farmlands were abandoned in the late 1800s. Ecologists monitored these lands for over 100 years, noting how the vegetation and the animals that it supported changed. The results of this study are illustrated in Figure 4.4.1.

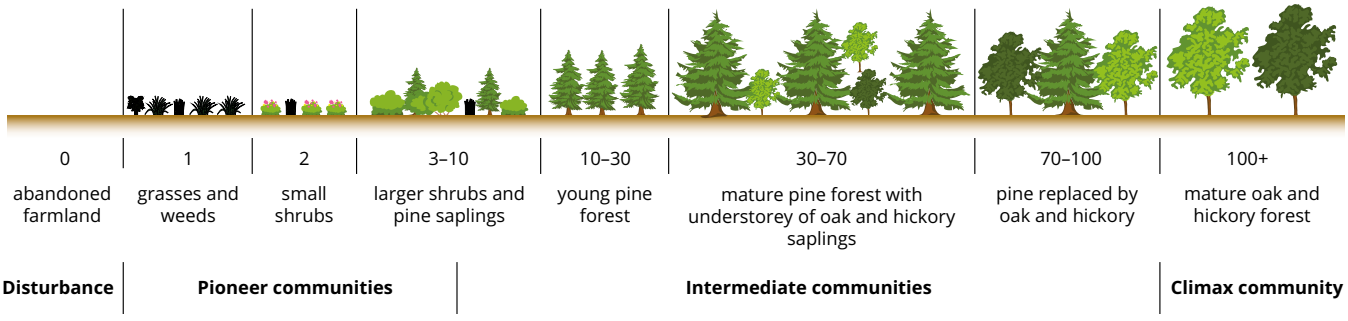


FIGURE 4.4.1 The succession from cleared, abandoned farmland to a climax community of mature oak and hickory forest took close to a century.

Over a period of time, a series of communities form. These successive communities are each called a **sere**. Each sere replaces the previous one until finally a stable community, the **climax community**, is formed.

The first sere is the **pioneer community**. The organisms of the pioneer community, the pioneers, generally have traits that fit them to live in harsh environments. These environments are often very exposed and lack nutrients and water.

Plants are unable to use nitrogen from the air. They must take in their nitrogen as nitrates. Plants rely on bacteria that absorb atmospheric nitrogen and produce nitrates, which the plants then use for their nitrogen requirements.

Pioneer organisms are the opportunists. They take advantage of the lack of competition in new or disturbed environments. Pioneer plants often have symbiotic relationships with soil bacteria that fix nitrogen from the air. The soils that the pioneers are colonising are generally poor in nutrients. This association provides them with nitrogen, which is a fundamental need as it is required for protein synthesis.

Lichens are often one of the early colonisers of bare rock. Lichens are a fungus and a photosynthetic organism living in a mutualistic relationship. In many cases, the photosynthetic organism is a cyanobacterium capable of nitrogen fixation. Pioneers typically used *r*-selected reproductive strategies (Module 2.1).

Pioneer plants are often shade-intolerant and do not compete well with larger plants that have more extensive canopies.

Pioneer plants usually arrive early in the establishment of new ecosystems but, as the ecosystem becomes more complex, they are out-competed by later arrivals and decrease in abundance or disappear altogether from the ecosystem. The pioneers alter the environment enough to allow the establishment of species that are less tolerant to the harsh conditions tolerated by the pioneers.

The organisms, especially the plants, of the climax community have very different traits to those of the pioneers. Organisms of the climax community typically adopt more *K*-selected reproductive strategies. Table 4.4.1 compares some of the traits of pioneer and climax species.

The compensation point is the point where photosynthesis and respiration occur at exactly the same rate. Below the compensation point, the plant will be releasing carbon dioxide. Above the compensation point, the plant produces more oxygen than it uses.

TABLE 4.4.1 Comparison of traits of pioneer and climax community species

Characteristic	Pioneer species	Climax species
photosynthesis/respiration compensation point	relatively high light intensity	relatively low light intensity
efficiency at low light	low	high
photosynthetic rate	high	low
seed number	high	low
seed dispersal distance	large	small
seed viability	seeds remain viable for relatively long periods of time	relatively short periods of time
plant size at maturity	small	large
structural strength	low	high
growth rate	rapid	slow
growth curve pattern	J-shaped	S-shaped
maximum life span	short	long
germination rate of seeds	rapid	slow

Sun-tolerant plants, such as those that act as pioneers, are better able to undergo photosynthesis when light intensities are high. Conversely, the shade-tolerant plants that form climax communities are better able to photosynthesise when light levels are lower. They reach the compensation point at a lower light intensity (Figure 4.4.2).

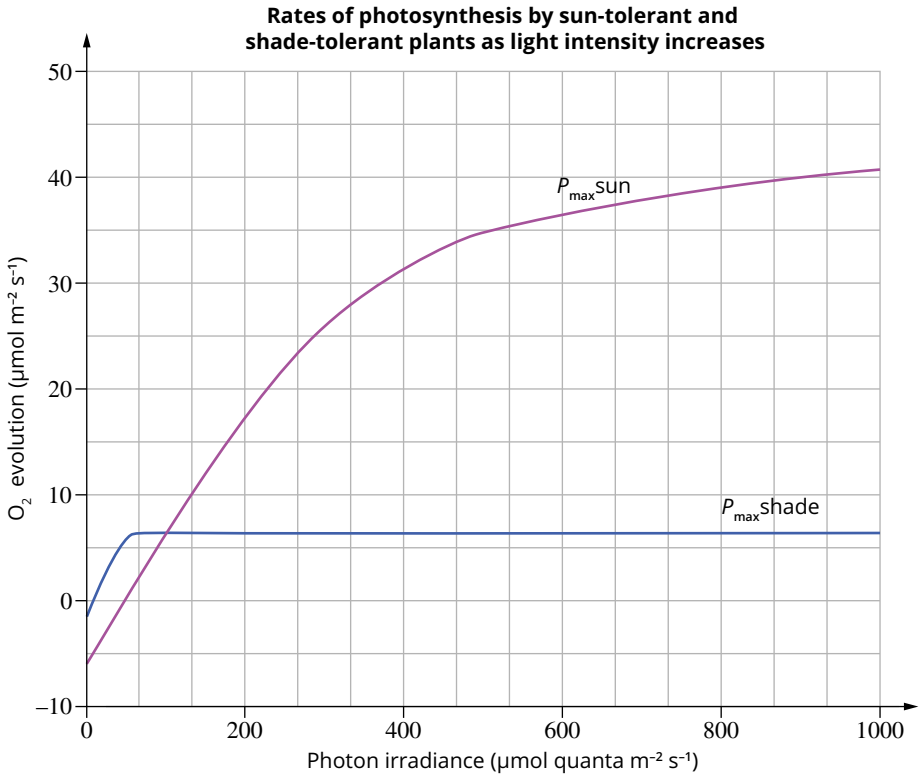


FIGURE 4.4.2 Sun-tolerant plants, such as those that act as colonisers, photosynthesise at a higher rate in full sun than the shade-tolerant plants that make up a large proportion of climax communities. The shade-tolerant plants photosynthesise more effectively in low-light intensities.

The succession from a pioneer community to a climax community can be observed in various places. Examples of succession include:

- the zones of the mangrove swamp and saltmarsh, where each of the series can be observed simultaneously
- a lake undergoing eutrophication, which must be observed over a period of time
- new land forming, such as the island of Surtsey in Iceland
- land that has been disturbed by human activity or natural disaster.

Mangrove swamps and saltmarsh

Estuarine ecosystems are of high ecological value. They are often inhabited by seagrasses and mangroves. They have high levels of biological productivity and provide a nursery environment for many marine species, including crustaceans and many fish. Loss of seagrass and mangrove swamps has been associated with the depletion of fish stocks, as fewer juvenile fish survive to adulthood. Estuaries are dynamic environments that are often responsible for the formation of new land.

A mangrove swamp or saltmarsh shows **zonation** associated with levels of tolerance to salt and waterlogging (Figure 4.4.3). Each zone represents a sere in the succession from bare, water-covered sand to forest.

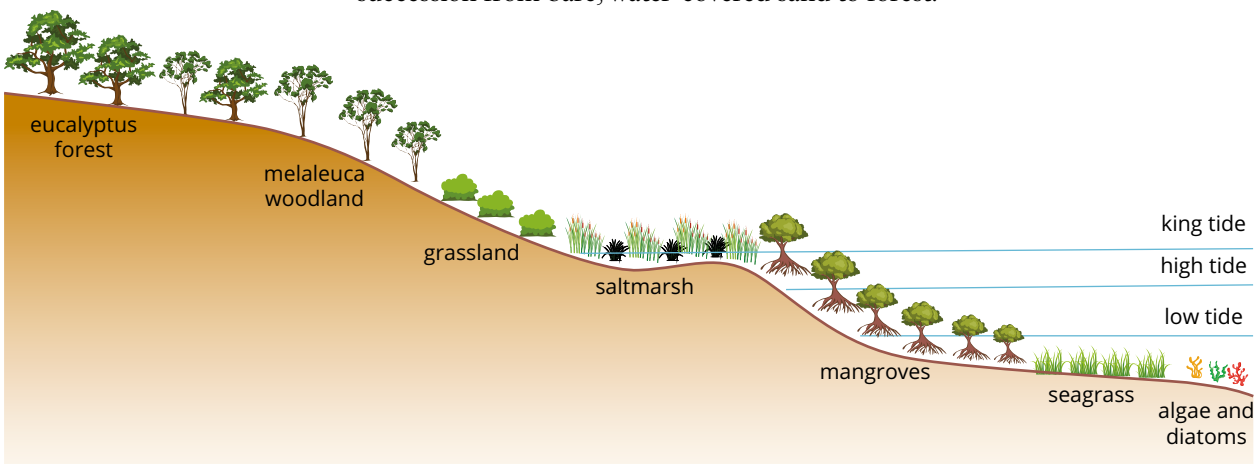


FIGURE 4.4.3 Coastal succession is shown by a saltmarsh and mangrove ecosystem that starts with algae and seagrass and ends with eucalypt forests.

The coastal community in Figure 4.4.3, which begins with algae and diatoms and ends with eucalypt forest, is a classic example of succession. Succession begins with algae and diatoms that secrete substances that hold sand grains together. This provides a suitable substrate for the growth of seagrasses. The seagrasses are land plants that have become adapted to living in marine conditions. They are true vascular plants, with a root system that helps consolidate the sea bed and trap sediment washed from the land (Figure 4.4.4). As the seagrasses die, they enrich the sandy bed with organic matter and improve the soil.

FIGURE 4.4.4 Seagrasses consolidate the sea bed, add nutrients to the sandy bottom, provide shelter and are a food source for many animals, including the endangered green sea turtle.

As the sea bed is consolidated and sediments and nutrients build up, the water becomes shallower and is exposed to the air through parts of the day. This environment is no longer suitable for the seagrasses but is conducive to the growth of mangroves. By the time the mangroves begin to colonise, the sand of the sea floor has given way to soils that are muddy, dark and rich in nutrients. Mangroves are well adapted to the conditions in this region of the intertidal zone. As well as being nutrient-rich, the soils are high in salt and low in oxygen, due to high levels of decomposition.

Mangroves are highly tolerant to salt. They can cope with waterlogging and long periods of submersion, because they have special root structures, called pneumatophores, that act like snorkels and allow air to reach the cells of their roots (Figure 4.4.5). The extensive root systems of the mangroves trap sediments, and their constant shedding of leaves increases the nutrient levels in the soils. These adaptive physiological responses make mangroves ideal pioneer plants, as they can live in an environment which would be lethal to most other plants.

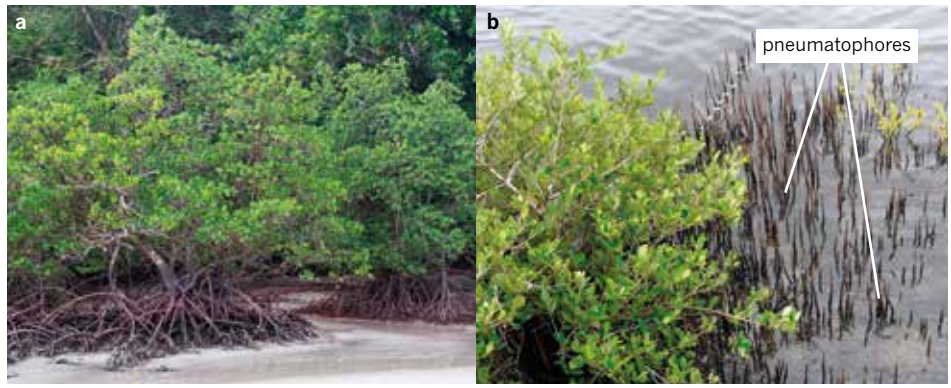


FIGURE 4.4.5 (a) Mangroves along the Daintree coastline have extensive root systems. (b) Mangroves can exist in the waterlogged coast because they possess pneumatophores that funnel air into the roots, keeping the plants oxygenated.

Mangroves change their environment over time by making and capturing more and more soil, causing the land to become higher and higher until finally the land is only inundated during the highest tides of the year or major storms. This creates an environment that is suitable for plants less able to cope with waterlogging. These new plants form a sere, which is the saltmarsh community.

The saltmarsh plants have physiological responses that adapt more tolerably to living in the air most of the time and out-compete the mangroves. Saltmarsh plants (Figure 4.4.6a) can adapt well to cope with the saline soils of the saltmarsh and the exposure to the drying environment of the coast. The highly saline environment is one in which water stress is a significant problem, so many saltmarsh plants are succulents (Figure 4.4.6b) and store fresh water in their leaves and stems. Communities like mangroves and saltmarsh, which are able to colonise harsh environments in which most organisms could not survive, are pioneer communities.

FIGURE 4.4.6 (a) Saltmarsh plants are highly adapted to life in water-stressed environments that experience constant exposure to sea spray and occasional flooding with sea water. (b) Many saltmarsh plants are succulents, which store water in their leaves and stems.

Further up the slope from the coast is usually the grassland sere. In the tropical parts of Australia, these can be several kilometres wide but they can also be only a few tens of metres. Grasses are better competitors than the succulents of the saltmarsh but are more sensitive to flooding by seawater. As the saltmarsh plants die and create more soil and the height of the land rises, flooding becomes more infrequent and the grasses are able colonise the edges of the saltmarsh. Over time, the grasses out-compete the saltmarsh plants and finally replace them.

The grasses further consolidate and add nutrients to the soil until conditions have improved sufficiently to allow more salt-sensitive species, like paperbark or tea trees (*Melaleuca* spp.), to survive and flourish. These plants form open woodlands consisting of medium-sized shrubs and small trees (Figure 4.4.7).

FIGURE 4.4.7 Paperbark trees form woodlands behind the grasslands in a coastal environment. These shrubs and small trees cope well with windy environments and shelter the areas further inland from wind and sea spray.

FIGURE 4.4.8 A eucalypt forest.

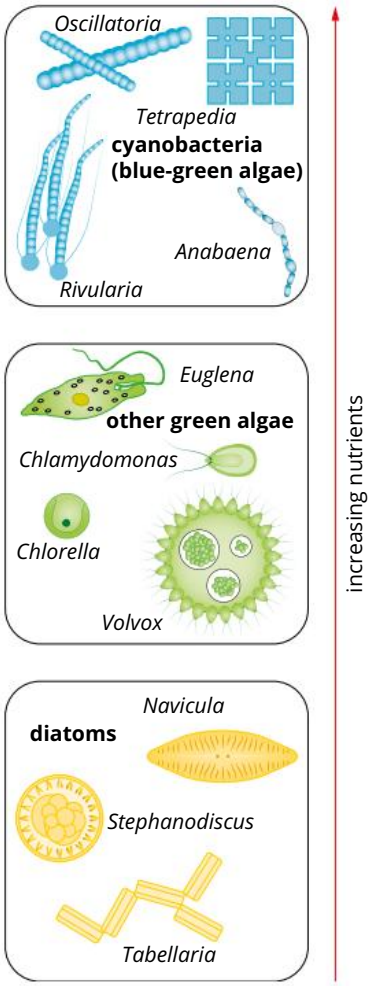


FIGURE 4.4.9 Algae are indicators of the nutrient level of a pond or lake. The lake changes as it ages. Desmids and diatoms are characteristic of young lakes, and green algae and cyanobacteria are characteristic of older, nutrient-rich lakes.

The last of the communities in many Australian coastal areas is the eucalypt forest. This sere is comprised of tall trees and may have a dense understorey (Figure 4.4.8). Climax communities like eucalypt forests remain stable until a major disturbance occurs, such as a fire, cyclone or land clearing.

In the case of coastal succession, the land gradually moves forward into the sea and each new sere gradually replaces the one that preceded it. Each successive community changes the surrounding environment enough to allow new species to colonise the area. Each community is characterised by a particular set of plants, which also support a specific set of animals.

Lake succession and eutrophication

Young, deep lakes generally have little algal and plant growth. As a lake ages, the abundance of floating phytoplankton and amount of plant growth on the edge and bottom often increases. Also, as the lake ages, it may become more productive as nutrients run off from the land and accumulate in the lake sediments.

The change of an aquatic environment from one that is low in nutrients to one that has very high levels of nutrients and is therefore more fertile and productive is called **eutrophication**. High levels of nutrients often support the development of algal blooms. The types of organisms present in the lake are good indicators of the stage of eutrophication because, as eutrophication progresses, the change in nutrient levels results in a change in the suite of organisms present.

Unicellular organisms, called desmids, are present in the early stages. These are replaced by diatoms, then green algae and finally by cyanobacteria (also called blue-green algae), as shown in Figure 4.4.9. Over time, the lake receives sediment washed in from the land. This may result in it gradually becoming progressively shallower.

As the lake becomes shallower, reeds grow along the edge in the shallow water. As more sediment collects, the lake gets smaller and the reeds grow further in. The stems and roots of the reeds trap more sediments and hasten the filling in of the lake. Behind the reeds, plants that tolerate waterlogging, like paperbark trees, grow behind the reed zone. As the trees get taller, they cast shade over the reeds, eventually killing them. Reeds are highly shade-intolerant. Eventually the lake fills in completely and the paperbarks cover the entire area (Figure 4.4.10). As the suite of plants changes, so do the animals that are supported by them.

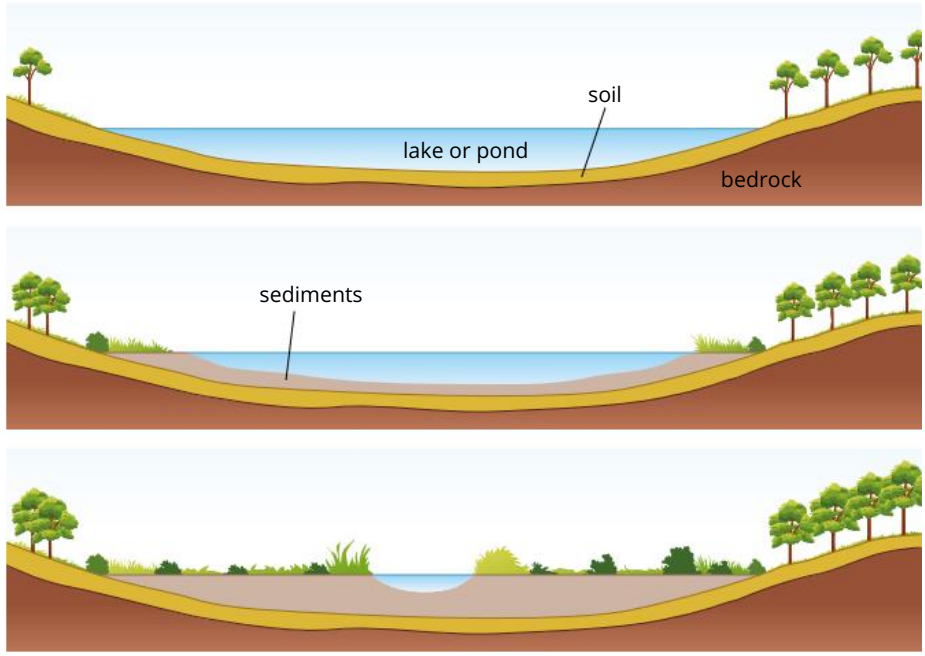


FIGURE 4.4.10 One example of succession is the gradual conversion of a lake to a paperbark woodland.

During the later stages of succession, the lake is likely to become **anoxic** (oxygen-depleted). As the waters become shallower, organic matter builds up in the lake basin. This organic matter is broken down by decomposers, but the process of decomposition requires oxygen. As the decomposers use up the oxygen, it becomes unavailable to other organisms. Exposure to oxygen concentrations of less than $2\,mg\,L^{-1}$ results in the death of most aerobic organisms. This leaves only anaerobic bacteria able to survive. The process of oxygen depletion occurs faster in warmer temperatures, as oxygen is less soluble in water at higher temperatures (Figure 4.4.11).

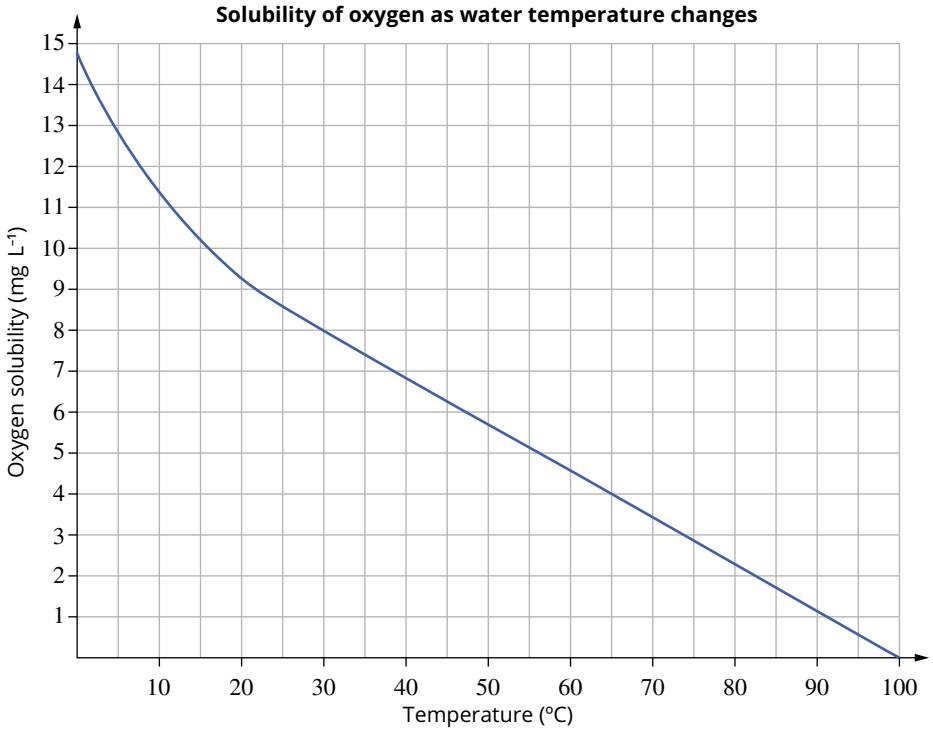


FIGURE 4.4.11 The change in solubility of oxygen as water temperature varies.

The principal cause of change in the lake is a physical process: the filling of the lake with silt and other sediments. The organisms in the lake also contribute to the process of environmental change through trapping silt and adding nutrients as they die and decompose.

The build-up of organic materials, which leads to oxygen depletion, can also happen in estuaries and other aquatic environments where there is a large nutrient inflow but water moves very slowly, if at all. Slow-moving water does not mix the water with air. This keeps oxygen levels low. In contrast, fast-moving water allows mixing of oxygen from the air with the water, which stops the development of the anoxic conditions associated with eutrophication.

Primary succession

Primary succession occurs when a bare area is colonised by organisms for the first time. Examples of primary succession include:

- the gradual encroachment of seagrass into a bare sea bed, leading to the formation of new land and mangrove swamps
- the colonisation of bare lava flows or coral cays.

An example of primary succession: Surtsey, Iceland

FIGURE 4.4.12 Sea rocket is a pioneer species that has fleshy leaves and stems which allow it to conserve water and establish itself on bare rock.

In 1963, the eruption of an undersea volcano 200 km off the southern coast of Iceland formed a new island. Eruptions continued until 1967 and have not recommenced. The formation of an entirely new and completely bare island gave biologists a unique opportunity to study primary succession. The new island was declared to be a nature reserve in 1965 and access was restricted. Scientists who visit the island are careful to avoid accidentally introducing any new species.

Species arrival and numbers were carefully monitored by scientists. The first species to arrive on the bare rock was sea rocket (Figure 4.4.12). Like with many pioneer species that inhabit salty, water-deficient environments, sea rocket has fleshy leaves that store water when it is available. Soon after the sea rocket, other small pioneer plant species became established: sea sandwort, sea lyme grass and oyster plant.

Plants initially arrived on Surtsey as a result of wind and water currents. Plants that colonised the island later were carried there by birds. By 1980, over 20 species of small plants were well-established, and by 1998 the first bushes appeared: tea-leaved willow. Figure 4.4.13 shows the increase in plant species on Surtsey from 1965 until 2015.

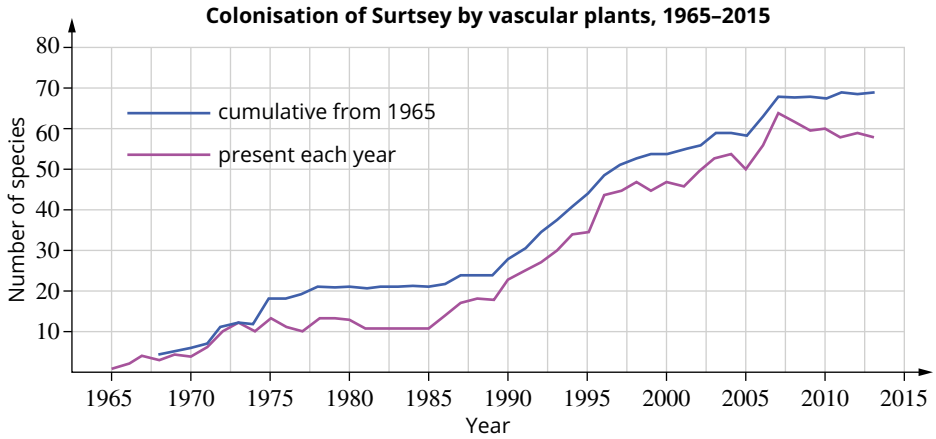


FIGURE 4.4.13 Colonisation of Surtsey by vascular plants between 1965 and 2015.

By 1984, birds had started nesting on the island and by 1985 a large seagull colony was established. Once the gull colony was well-established, the birds became a major factor in the increase in plant species on the island. Figure 4.4.14 shows the contributions of wind, water and birds to the species diversity of Surtsey.

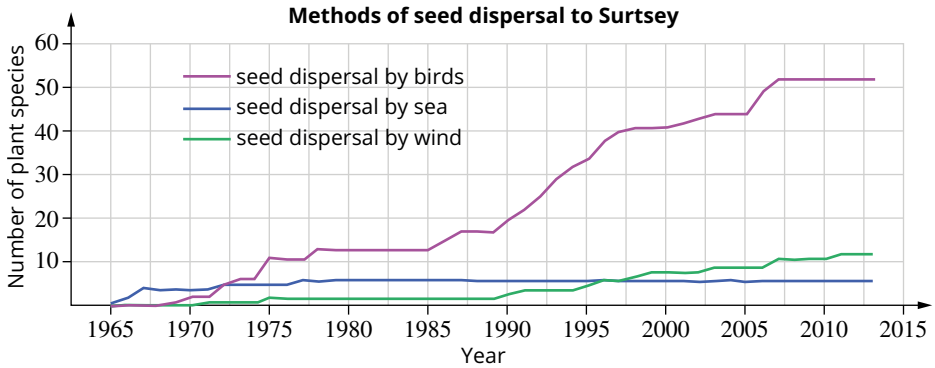


FIGURE 4.4.14 Methods of seed dispersal to Surtsey. Dispersal increased significantly once birds were well-established, shown by the sharp rise in the number of plant species beginning in 1985.

The establishment of the gull colony also saw an increase in the enrichment of the soil through bird droppings (Figure 4.4.15). As a result, the area near the colony is the most heavily vegetated area today. The formation of increasingly nutritious soil has allowed more plant species to become established. This is occurring at the rate of about 2–5 species per year. As new species become established, some of the earlier arrivals are declining in abundance.

Along with the plants and birds, there have been other arrivals on Surtsey. Several species of insects were probably carried to Surtsey by strong winds. As these insects are also found in Iceland, it is likely that is their origin. Once plants were established, herbivorous insects carried to the island had an available food source and were able to become established. The herbivorous insects then became a food source for carnivorous insects. Seals and several species of birds have breeding colonies on the island today. The arrival of seals attracted killer whales into the seas surrounding the island, as the whales feed on the seals.

Surtsey continues to act as a natural laboratory to explore the processes of succession on land that was originally completely devoid of life.

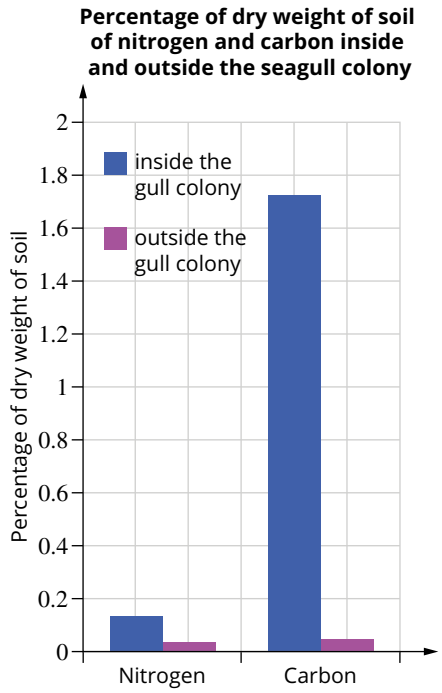


FIGURE 4.4.15 Nitrogen and carbon levels in the soil are much higher in the area occupied by the seagull colony. Higher levels of soil nutrients allow the establishment of a larger range of species.

Secondary succession

Secondary succession occurs in areas that were once vegetated but have been disturbed. This may be after human activities, such as clearing the land for farming and then abandoning it, or natural events like major forest fires. After the disturbance, plants and animals begin to reclaim the area.

For example, if a eucalypt forest is cleared for farmland and then abandoned, plants and animals will reclaim the area. Native herbs, bracken fern or weeds will be the first plants to recolonise the cleared area. Pioneering plants include plants that produce many seeds, which are transported into the area by wind, water or animals. The pioneers thrive in the high light intensities of open areas.

Later, the seeds of shrubs and trees are carried into the open area from surrounding eucalypt forests. As the trees grow, the expanding canopy changes the environment. The habitat becomes more complex, with more shaded areas and fewer sunny patches. Shade-intolerant herbs and weeds disappear. Animals, such as birds and possums, invade the upper layers of the forest as food becomes available. Litter builds up and detritivores increase in diversity and levels of activity. Eventually, a stable climax community forms (Figure 4.4.16).

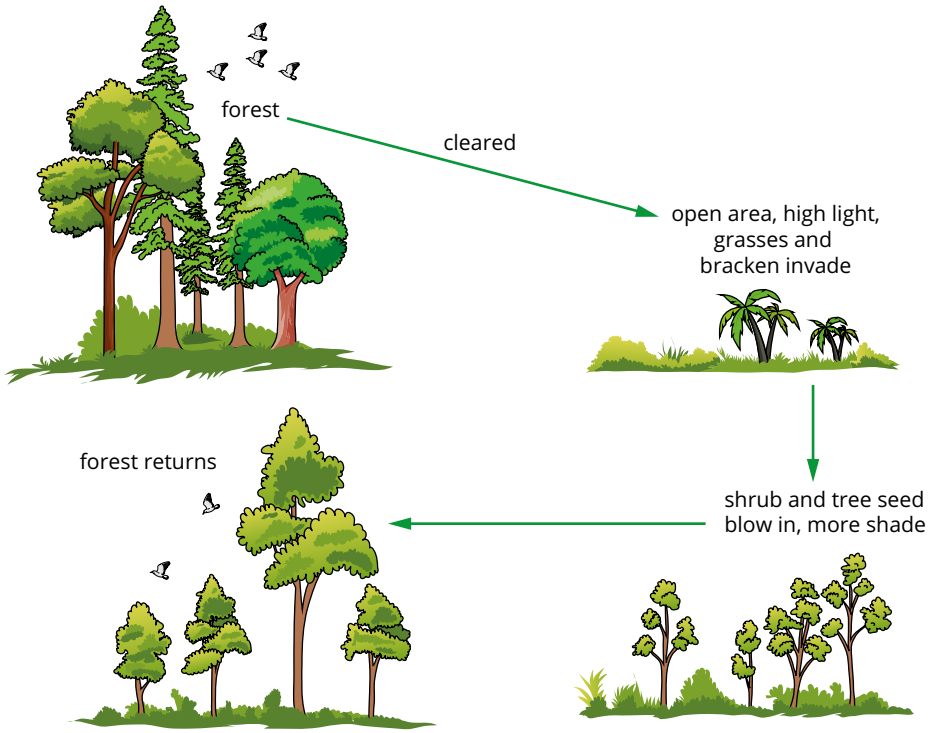


FIGURE 4.4.16 The regeneration of a forest following a disturbance occurs in a series of steps during which there is a gradual change in the composition of the community of organisms living in the area.

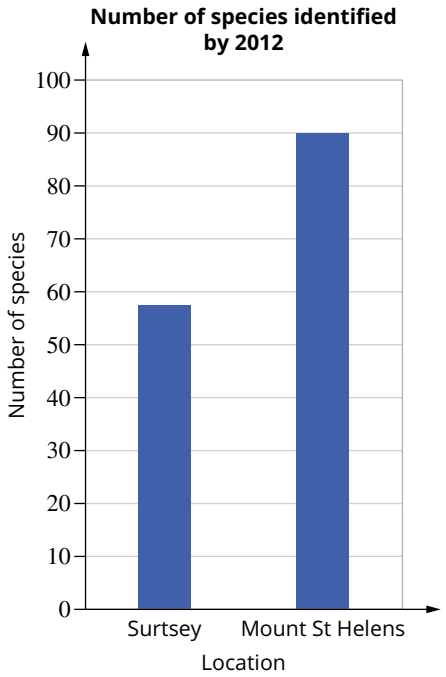


FIGURE 4.4.17 Number of species present on Surtsey and the slopes of Mount St Helens in 2012.

Primary succession versus secondary succession

Secondary succession is usually much faster than primary succession. Because there was a previous community, the soils involved in secondary succession are generally higher in nutrients than the bare ground that commences primary succession events. There may also be seeds lying dormant in the soils of secondary succession events or root stocks, bulbs and corms in the soil, which will shoot and grow new vegetation quickly.

One study compared succession on Surtsey with the revegetation of the area around Mount St Helens in the USA after its eruption in 1980. Both examples of succession occurred as a result of volcanic activity, but the area of Mount St Helens had long been vegetated whereas Surtsey was entirely new. The comparison of the two succession events shows the differences between primary and secondary succession.

Sampling plots were established in both areas and monitored to identify the number of species present. Permanent plots were established on Surtsey in 1990, prior to which individual plants were counted, while those on Mount St Helens were established in 1982 and monitored until 2010. Monitoring of sample plots continues on Surtsey today and there is no plan to stop doing so.

Although most areas on Mount St Helens started out completely devoid of plants, by 2012, several plots supported conifer trees. In the same year on Surtsey, most of the vegetation was low herbaceous plants and there were a few small bushes, despite Surtsey coming into existence in 1963. Figure 4.4.17 shows the results of a species count in both places taken in 2012.

The significant difference between the two sites is related to the different prior environment. In the case of Mount St Helens, where secondary succession occurred, there had previously been many species present. Despite the eruption, there were seeds and cuttings that survived and started to regrow. Also, the devastated area of Mount St Helens was surrounded by established communities that provided seeds and from which animals could easily migrate. Finally, the soils in the Mount St Helens area were deep and high in nutrients because they had been formed over hundreds of years prior to the eruption. In contrast, Surtsey, where primary succession was occurring, was bare rock with no soil and the nearest source of plants and animals was Iceland, more than 200 km away.

USING THE FOSSIL RECORD TO IDENTIFY ECOLOGICAL CHANGE

Ecosystems have been in constant change since life first appeared. Studying these changes and identifying their causes involves, among other things, the study of fossils. Fossils are used in many different ways to identify changes in both biotic and abiotic factors in the past and determine what caused the changes.

Researchers base many of their ideas about past ecosystems on the idea that 'the present is the key to the past'. This concept states that the adaptations of organisms are related to the environment in which they live and that many adaptations appear again and again. An organism that lives in an aquatic environment will have features that fit it for that environment.

If you find a fossil shaped like a modern fish in a layer of rock, you can infer that the environment at that time was an aquatic one. If you find many fossilised teeth with adaptations for eating grass, you can hypothesise that the area was a grassland at that stage of the Earth's history.

Fossils as an indicator of atmospheric gas composition

Rising levels of CO_2 in the atmosphere in modern times has caused scientists to delve into the past to obtain data about the probable effects on future ecosystems. In order to do this, the CO_2 levels of past atmospheres must be determined. One method used to determine past atmospheric CO_2 concentration is the study of stomatal density.

For plants, stomatal number and opening is a balancing act between acquiring enough CO_2 to support photosynthesis and minimising water loss through transpiration. As temperatures rise due to increased atmospheric CO_2, transpiration becomes more of a problem but diffusion rates for CO_2 increase due to a steeper concentration gradient. Understanding this balancing act, plant biologists hypothesised that as CO_2 concentration rose the number of stomata would decrease. Research was undertaken to test this hypothesis. In one study, plants were grown in CO_2 levels lower than the present (180 ppm), at present-day concentration (450 ppm) and at levels predicted to be possible by the end of this century (1000 ppm). The plants' responses, the number of stomata per mm^2, was determined. Results for two of the species tested are shown in Figure 4.4.18. As the CO_2 concentrations increase, the stomatal density decreases, and the relationship appears to be fairly linear.

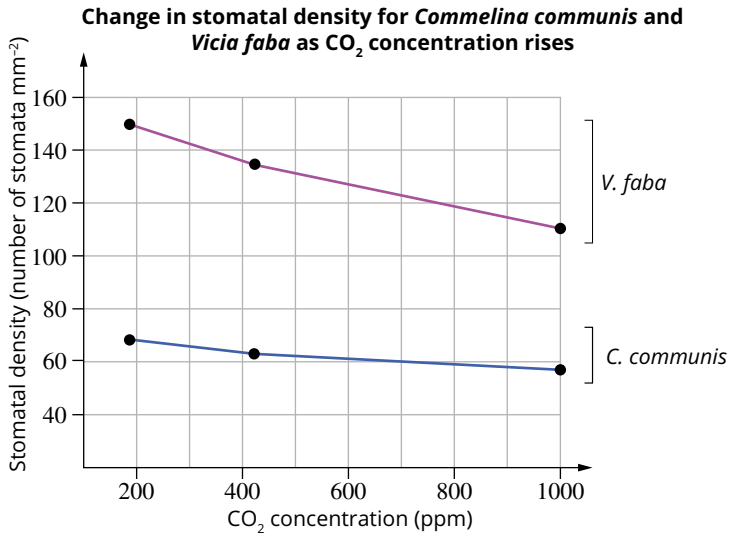


FIGURE 4.4.18 Stomatal density in increasing levels of CO_2 shows an inverse linear relationship that can be used to estimate CO_2 concentrations of ancient environments.

Using this correlation, paeleontologists have examined fossil leaves, calculated the stomatal density and used the data to estimate the CO_2 concentrations of the atmosphere in the past. As Figure 4.4.18 shows, the natural variation between species at the same CO_2 concentration is considerable, so scientists need to compare stomatal densities between plants of closely related species grown at a known concentration with those grown in the unknown CO_2 concentration. In most cases, the fossil species of plant are long extinct, so scientists use their nearest living equivalent (NLE). The NLE is generally a related species that shows similar ecological and/or structural features.

In one study used to estimate Eocene CO_2 atmospheric concentrations, fossil plants belonging to the genus *Litsea* were compared for stomatal density with modern NLEs. The modern NLEs were specimens present in museum and botanical collections that had been collected from plants during the early 1800s, under pre-industrial revolution CO_2 concentrations. The data is shown in Table 4.4.2.

TABLE 4.4.2 Mean stomatal densities of modern and Eocene members of the genus *Litsea*

Species	Age	Mean stomatal density (mm^{-2})	Standard deviation	Number of samples tested
Litsea hirsuta	Eocene	315.1	37.9	57
Litsea bournensis	Eocene	337.5	47.4	56
Litsea edwardsii	Eocene	271.3	33.6	51
Litsea sebifera	Modern	406.9	33.2	131
Litsea fuscata	Modern	454.0	41.9	30
Litsea stocksii	Modern	407.2	47.6	132

The data show that the mean stomatal densities were lower for the plants from the Eocene. This has been correlated to a CO_2 concentration in the air of between 3.2 and 5.8 times the pre-industrial revolution levels.

There are limitations with this form of data. There is considerable natural variation in the stomatal density of different species, as can be seen in Table 4.4.3. Stomatal density of *Litsea fuscata* is significantly higher than that of either *Litsea sebifera* or *Litsea stocksii*. Also, not all species respond in the same way to changes in CO_2 concentration. A study involving many species subjected to growth in raised CO_2 found many species that did not show raised stomatal densities.

TABLE 4.4.3 Percentage of species in various subclasses of plants that respond to a CO_2-enriched environment with an increase in stomatal density

Subclass	Percentage of species which show an increase in stomatal density
Dilleniidae	20
Caryophyllidae	30
Asteridae	27
Hamaelidae	13
Rosidae	20

Other limitations with the use of this method include plant responses to different environments. The altitude at which a plant grows has been shown to influence stomatal density. The pressure of CO_2 at higher altitudes is lower and diffusion rates are slower, which would cause plants to have a higher stomatal density than plants at lower altitudes. Finally, light availability and water availability while the plant is growing have also been shown to influence stomatal density.

CASE STUDY 4.4.1

Fossils and the environment: Using fossil teeth

In one study, the enamel of fossil teeth deposited during the period of the Miocene/Pliocene boundary (between 6 and 8 million years ago) were analysed to determine their ratio of two isotopes of carbon: carbon-12 and carbon-13. These two isotopes are incorporated during photosynthesis to a different extent by plants using C_3 and C_4 photosynthetic pathways: C_4 plants have a lower proportion of carbon-13. The carbon is utilised by, and incorporated into, animals that eat the plants.

In the C_3 photosynthetic pathway, carbon dioxide is initially incorporated into 3-phosphoglyceric acid which contains three carbon atoms. In the C_4 pathway, carbon dioxide is initially incorporated into oxaloacetate, which contains four carbon atoms. Ultimately, both pathways result in the production of glucose.

Eighty-five per cent of modern plants use the C_3 pathway. This group includes most cereal crops, including rice, wheat, soybeans and peas, some grasses and most tree species. About 3% of modern plants, including a few crops such as sugar cane, sorghum and maize, some grasses and most sedges, use the C_4 pathway.

Analysis of modern plants shows that plants using the C_3 pathway contain a greater proportion of carbon-13 than plants using the C_4 pathway.

Fossil teeth from the period from 8 to 6 million years ago contain gradually decreasing levels of carbon-13. This indicates that the animals had an increasing proportion of C_4 plants in their diet. C_4 plants photosynthesise more efficiently than C_3 in lower carbon dioxide concentrations. Using these two observations, scientists have postulated that this gradual change in diet of the animals indicates a decreasing concentration of atmospheric carbon dioxide.

As with all hypotheses about conditions in the past, other interpretations may be equally valid and final conclusions should not be based on only one piece of data. The shift to C_4 metabolism is also correlated with falling mean atmospheric temperatures, as C_3 metabolism is more efficient at higher temperatures (like that found in the tropics today).

As with all methods of determining past environments using fossil evidence, this method should not be used on its own. Confidence in conclusions comes from using a variety of different methods and obtaining a range of data that supports the same hypothesis.

Review

1. Identify at least two assumptions that the conclusions of this study rely upon.
2. Discuss the possible effect of a rise in CO_2 levels on the sugar cane industry in Queensland.
3. Contrast conditions 8 million years ago with those 6 million years ago.
4. From the data related to carbon-13, infer how competition between C_3 and C_4 plants altered their abundance through the period from 6 to 8 million years ago.

Microfossils as an indicator of change in temperature

Microfossils are the fossilised remains of micro-organisms and other microscopic organic material. Diatoms, dinoflagellates and pollen grains commonly form microfossils.

Dinoflagellates and diatoms are tiny unicellular members of the Protista kingdom (Figure 4.4.19). They are an important constituent of the phytoplankton found in the upper layer of the ocean. There are also freshwater dinoflagellates and diatoms that inhabit the surface layers of lakes and streams. At times in their life cycle, dinoflagellates form tough encapsulated cysts that are resistant to decay, so they often fossilise very well. These cysts sink to the floor of their aquatic environment and are preserved. Diatoms have a cell wall made of silica, which is highly resistant to decay, so they also fossilise well.

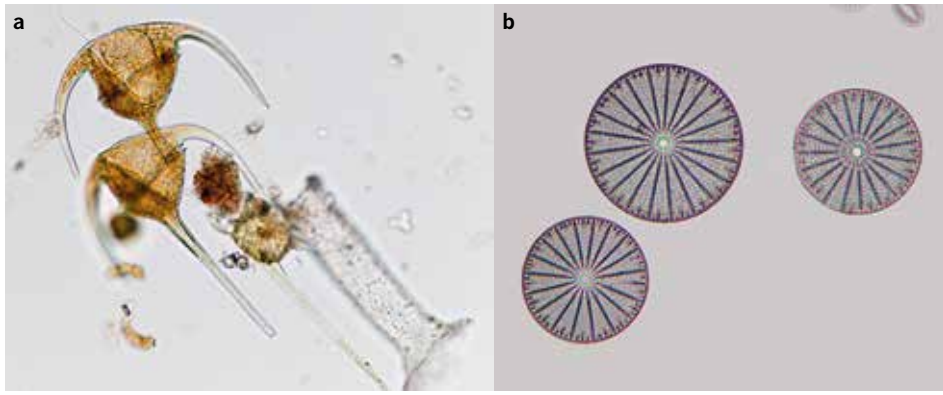


FIGURE 4.4.19 (a) A dinoflagellate of the genus *Ceratium* and (b) a diatom of the genus *Arachnoidiscus*, viewed under a light microscope (× 100).

The law of superposition of sediments says that unless there have been major geological disturbances the layers of soil or rock closer to the surface are younger than those below. This rule allows researchers to build up a picture of the past in chronological order. When using such cores, the researcher must be aware that geological disturbances may have disrupted the order of the sequences and that the core may not represent a comprehensive picture of the area, as no deposition may have taken place at some periods or erosion of previously deposited layers may have removed some of the layers. Even with these limitations, rock cores provide useful data about ancient ecosystems.

A core is produced when soils or rocks are drilled. As the drill goes deeper, the tubes of rock or soil become older. These cores can then be arranged in order and analysed. The microfossils can then be identified after microscope slides of sections of the rock are prepared and examined. A series of rock core drilled in Australia are shown in Figure 4.4.20.

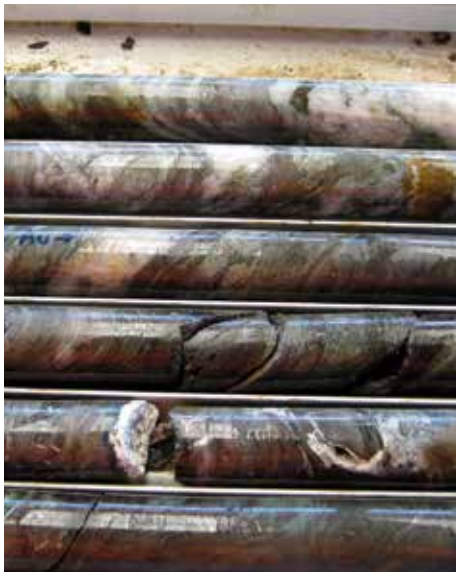


FIGURE 4.4.20 Rock core drilled from ancient rocks of Australia. Microscope slides of thin sections from these cores can be analysed to identify microfossils.

Dinoflagellates and diatoms found in rock cores can tell scientists two things about the ancient environment of an area. First, their presence indicates the existence of an aquatic environment and the species present will indicate whether that environment was freshwater or marine.

Second, many species are highly sensitive to temperature, so the suite of dinoflagellate and diatom species present at any particular time indicates the temperature of the water during that period. The graphs in Figure 4.4.21 illustrate the ideal temperature ranges of four species of diatom. As can be seen, the ideal temperatures vary considerably.

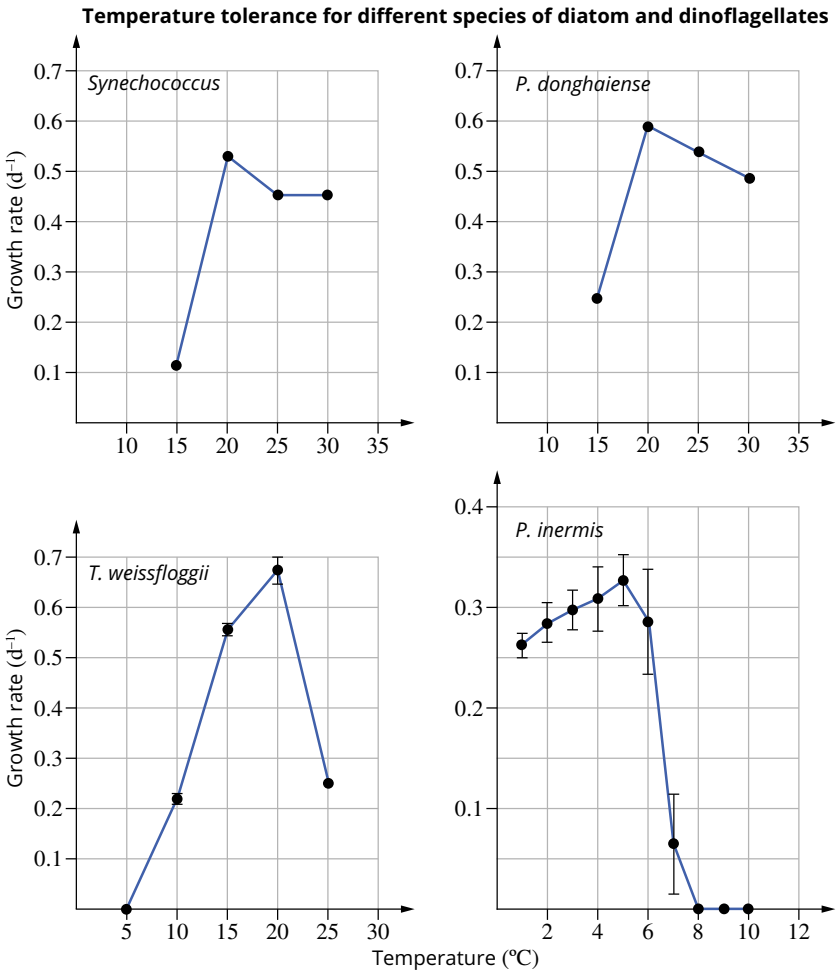


FIGURE 4.4.21 The tolerance ranges for temperature varies between different species of diatom and dinoflagellates.

The mix of species of diatoms and dinoflagellates in an area at any one time are an indicator of the water temperature. Examination of the fossil species composition, and how it changes, along with the use of radiometric dating techniques to provide ages for the rock samples, can be used to build up a picture of how the temperature of the Earth has changed through geological time.

Microfossils as an indicator of change in habitat

Current evidence shows seed-bearing plants first appear in the fossil record during the late Devonian, around 360 million years ago. With the arrival of the seed-bearing plants came pollen. Due to its tough outer case, pollen fossilises well. Pollen grains also appear in shapes that are unique to particular species. Figure 4.4.22 illustrates a few different shapes, sizes and ornamentations of pollen grains.

A core can be taken in a particular area and the mix of pollen grains examined. Different mixes of species indicate different environments. Lots of grass pollen indicates a grassland and large amounts of cycad pollen indicate a cycad forest.

FIGURE 4.4.22 Pollen grains (× 500) from several different species: sunflower (small pink spheres), morning glory (large green spheres), hollyhock (large yellow spheres), lily (green, bean-shaped pollen), primrose (red, Y-shaped pollen) and castor bean (small pale-green spheres).

HUMAN ACTIVITY

When humans first evolved, our numbers were small and we fitted into ecosystems in the same way as any other omnivore. Humans ate some animals and plants, though it is inferred that, as hunter-gathers, early humans generally moved on to new areas before they made large impacts on the ecosystem. Today, our huge population and large demands for food and other resources means that we have a more significant impact on the environment than ever before.

As the human population continues to increase, the demands we make on the environment will inevitably rise. Projections for the population of humans vary between 9.5 and 13.3 billion by 2100 (Figure 4.4.23).

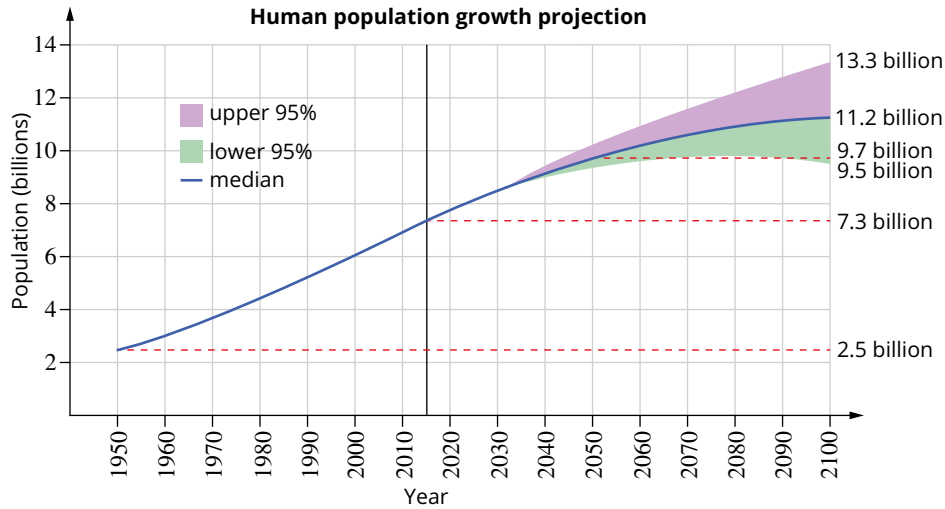


FIGURE 4.4.23 Projections of human population growth.

Human activities that cause significant ecological change include:

- land clearing
- rising carbon dioxide
- introduction of new species
- spread of disease
- over-fishing and hunting
- genetic modification and selective breeding
- planting of monocultures
- nutrient cycling.

Land clearing

Clearing of eucalypt woodland and forest for agriculture has had the greatest effect on the loss of species in Australia. Of the endangered plant species in Australia, about 85% are under threat because of agriculture and grazing. In the south-west of Western Australia, where more than two-thirds of the region (190 000 km^2) is farmland, 13 native mammal species have become extinct since the arrival of Europeans.

Birds are also affected by the replacement of native vegetation with crops such as wheat. Some parrots, such as the galah, little corella, long-billed corella and inland red-tailed black cockatoo, feed on the cereal crops and seeds of accompanying weeds. These birds have flourished, and the galah has reached pest proportions in some areas. However, over the same time period, other parrots, such as Major Mitchell's cockatoos, are experiencing difficulty. Although it has sufficient food because it eats a wide variety of seeds, including wheat grain, the Major Mitchell's cockatoo is territorial and a breeding pair will not usually tolerate another pair within 1 km of their nest. Also, the Major Mitchell's cockatoo only nests in tree hollows, so old trees are a necessity (Figure 4.4.24a). Since the Major Mitchell's cockatoo's woodland habitat has been reduced to a patchwork of small areas scattered among the wheat farms, the number of breeding pairs of birds, and therefore the population, is declining (Figure 4.4.24b).

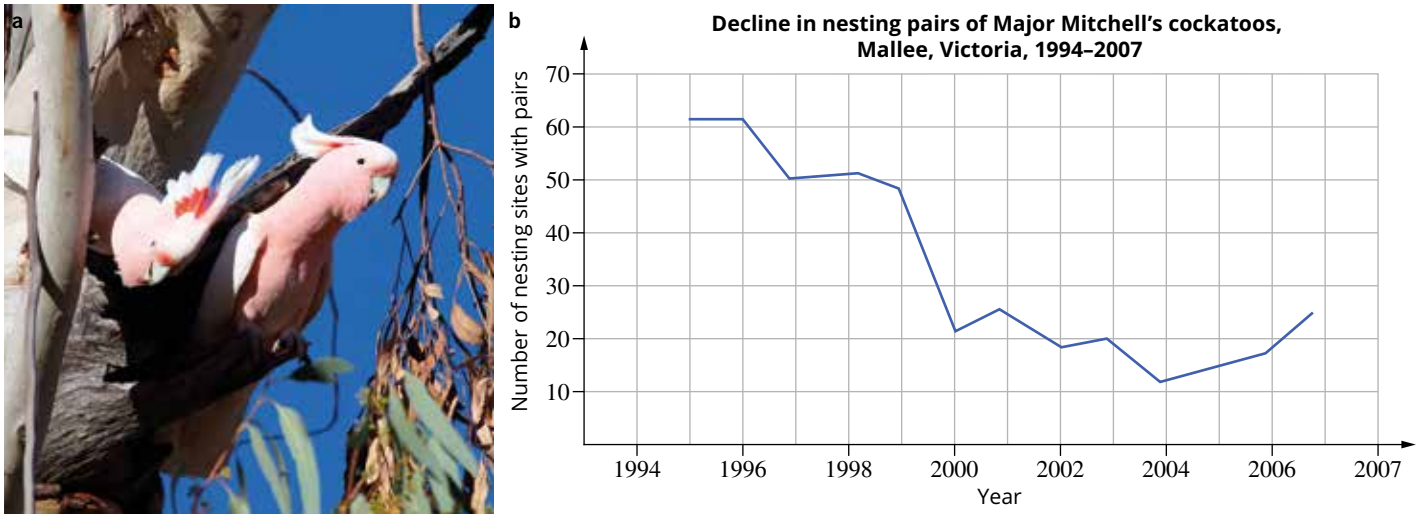


FIGURE 4.4.24 (a) Breeding pairs of Major Mitchell's cockatoos require old trees with hollows to build their nests. (b) The decline in numbers of breeding pairs of Major Mitchell's cockatoos in the Mallee, Victoria. The steep decline in 1998 was partially caused by poaching for the international bird market. In 2004, removal of galahs, which compete for nesting sites, from the area led to some recovery.

Habitat fragmentation

Land clearing also causes fragmentation of forests and other ecosystems (Figure 4.4.25). Fragmentation results in slower impacts. Each small ecological island may, in the beginning, seem to continue without major harm but more long-term effects are likely.

Loss of biodiversity through genetic diversity does not immediately impact an ecosystem. However, over time, reproductive cycles and generations, loss of genetic diversity can lead to the loss of species and result in simplification of food webs. Moreover, the magnitude of ecosystem change due to human impact varies depending on the extent and range of fragmentation as well as the type of organisms living in the fragmented habitats. For example, an ecosystem that is broken into larger fragmentations that are closer together and contain highly mobile organisms may be less affected by human impact than an ecosystem that is broken into very small fragments that are greatly dispersed and contain sedentary organisms

These small isolated islands of habitat do not fully replicate the large and unfragmented habitats that existed before land clearing occurred. There is a much greater area of edge and a much smaller area of inner habitat. The edges of a woodland offer different conditions (for example, exposure to wind, sunlight and particles in the air) to the inner areas and therefore support different species. Species with physiological adaptive responses suited to the edge are in greater abundance at the edge, with related distributions and realised niches. Species more suitably adapted to the inner areas are not so highly represented (Figure 4.4.26).

FIGURE 4.4.25 The consequence of land clearing for farmland is dispersed islands of forest that offer some habitat for native species, but which results in some species being separated into small isolated populations, with consequent effects on genetic diversity.

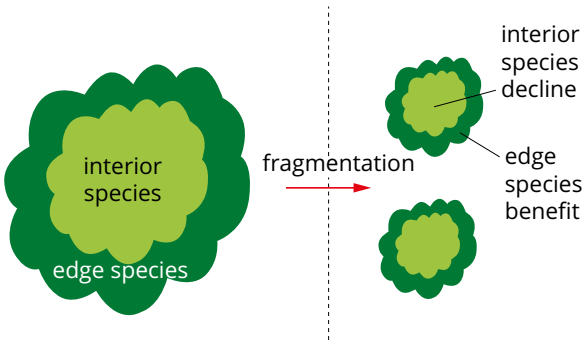


FIGURE 4.4.26 The edge of a forest has different conditions, such as more light, more variable temperatures and greater exposure to wind. This favours different species than the conditions in the interior.

Homozygosity is when the inherited pairs of genes (alleles) for a characteristic are the same and heterozygosity is when they are different.

As a result of the fragmentation, organisms like plants, which are unable to cross between the islands, become isolated into small populations. This can lead to inbreeding and the loss of genetic diversity. Homozygosity increases and heterozygosity decreases (one example of magnitudes is shown in Figure 4.4.27), leaving populations vulnerable to other environmental changes like new diseases (you will learn about heterozygosity in Chapter 7). Conversely, larger populations tend to have greater genetic diversity. Figure 4.4.27 shows the effect of an increase in population size on heterozygosity in one species of eucalypt. The magnitude change in heterozygosity as the population size increases is first significant, then levels off to plateau.

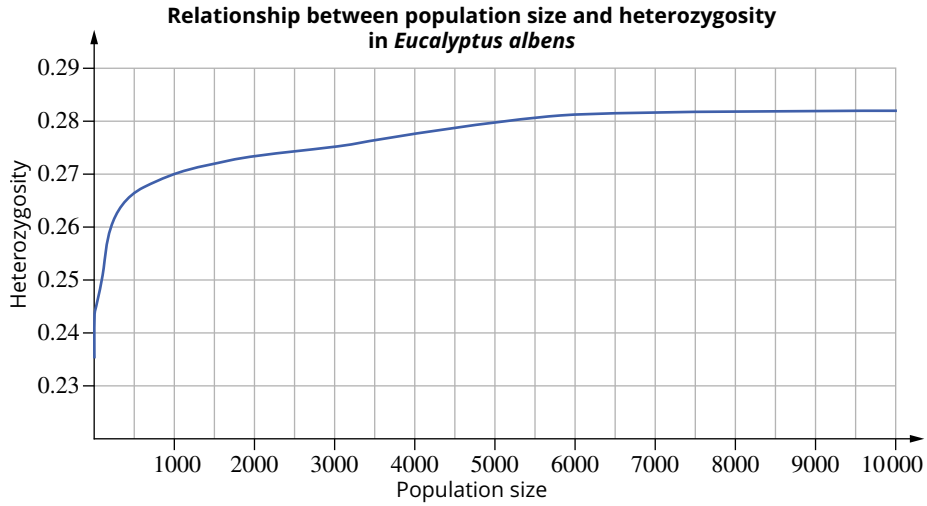


FIGURE 4.4.27 As the breeding population of *Eucalyptus albens* increases in size, there is greater variability in the genetics of the population. This is a typical pattern for most species.

Remember, while loss of biodiversity through genetic diversity does not immediately impact an ecosystem, over a period of time, reproductive cycles and generations, loss of genetic diversity can lead to the loss of species and result in simplification of food webs.

Dryland salinity

Land clearing, followed by the planting and irrigation of crops, also leads to degradation and increased salt in the soil. Increased levels of salt (largely sodium chloride), referred to as salinity, is a major problem in both land and adjacent aquatic ecosystems. Dryland salinity affects a considerable area of land across southern Australia. It is a direct result of clearing the land of native vegetation.

Much of Australia's soil naturally holds large amounts of salt, which originally came from the oceans. Palaeontological records show vast populations of fish and sharks, as well as marine reptiles and large marine organisms, in the deserts of Australia, especially western Queensland. Salt is also carried thousands of kilometres inland from oceans to arid regions. Out to sea, droplets of saltwater spray are thrown into the air by wave action. The wind blows the salt inland and rain deposits it on the soil. Eventually the salt moves down through the soil to the water table, where it builds up. The presence of salt is part of the history of the development of much of Australia's land ecosystems.

Plants take up water from the soil and lose it (through transpiration) from their leaves. This removal of water from the soil results in increased concentration of the salt that is left behind. For example, the groundwater in the wheat belt of Western Australia is sometimes even saltier than sea water. The salt is normally harmless to plants because it remains in the groundwater and subsoil, well below the depth to which the roots of shrubs and trees can reach (Figure 4.4.28). Problems arise when the native vegetation is cleared, the removal of water by deep-rooted shrubs and trees is decreased, and the level of groundwater rises. This process is hastened by irrigation, which adds water to the water table and causes it to rise more quickly.

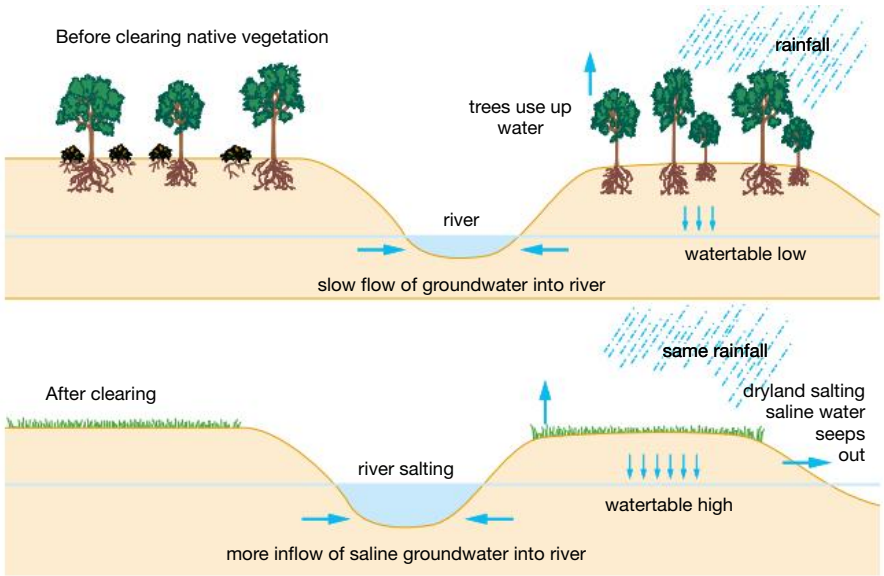


FIGURE 4.4.28 Clearing of vegetation causes dryland salinity. Rising groundwater brings salt to the surface.

As a result, the land becomes unable to support most species of plant leading to the loss of vegetation (Figure 4.4.29). Consequently, the duration of salinity effects are often quite long, as salt-tolerant vegetation needs to be planted and become established to begin to correct salt levels.

Native eucalypts have roots extending to depths of 15 m or more and are very good at extracting water. As the water rises, it brings dissolved salts closer to the surface. Once the salty water reaches a critical depth of 1.5–3.0 m, it rises to the surface by capillary action.

Finally, the planting of crops also results in large areas of plant monoculture. These ecosystems have very simple food webs. Fewer species of insect, and therefore insectivorous birds, live in ecosystems where there is only one type of producer.

FIGURE 4.4.29 Land irrigated using river water in the Murray–Darling basin is now too salty for crops and most other plants to grow.

Rising carbon dioxide

An increase in carbon dioxide levels in the atmosphere is one impact that humans have on the environment (Figure 4.4.30). As we burn fossil fuels (coal, oil and gas), the carbon that has been sequestered in the Earth's crust is released into the atmosphere as CO_2. It is hypothesised that this will cause the surface of the Earth to increase in temperature over the next centuries.

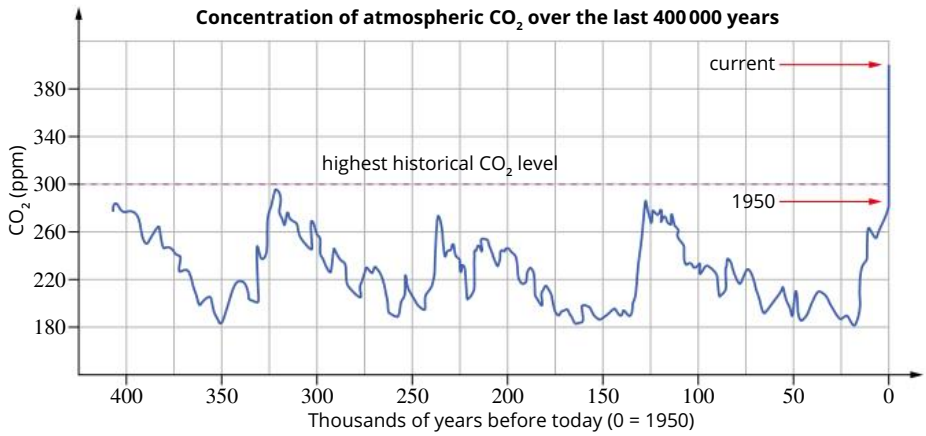


FIGURE 4.4.30 Concentration of atmospheric CO_2 over the last 400 000 years. Atmospheric CO_2 was determined by analysing air trapped in Antarctic ice cores. The ice was deposited over the last 400 000 years and, once formed, remained frozen until the present.

If carbon dioxide levels continue to increase, there will inevitably be effects on ecosystems. Changes in temperature, plant productivity, the carbon cycle and the water cycle (directly and indirectly related to transpiration) are examples of environmental changes that may be observed.

Studies of plant responses to higher carbon dioxide levels have shown that biomass increases are higher in plants when carbon dioxide levels are higher. In the study illustrated in Figure 4.4.31, the increase in productivity only occurred in 1998, which was a year with a relatively high rainfall (309 mm). The study continued into 1999 when rainfall was poor (17 mm). Growth in the 1999 year (not shown) was very small and exactly the same for both groups of plants, finishing at 30 mg after 270 days. These results suggest that the increased plant growth due to higher CO_2 concentrations is dependent upon there being sufficient rainfall.

The rise in productivity could imply that plants are benefiting from the rise in CO_2; however, other studies have shown that the plants have reduced nutritional value.

One series of studies examined important food crops: rice, wheat, soybeans, peas, maize and sorghum. It showed a significant reduction in the concentrations of zinc, iron and protein in all of the species (Figure 4.4.32). This reduction, if confirmed by future studies, could have detrimental effects on humans and other herbivorous species, as these elements are essential for maintaining good health.

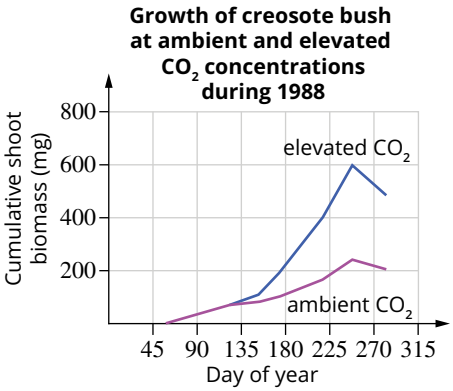


FIGURE 4.4.31 The effect of rising CO_2 concentration on *Larrea tridentata*. Ambient CO_2 levels are those of the present-day atmosphere.

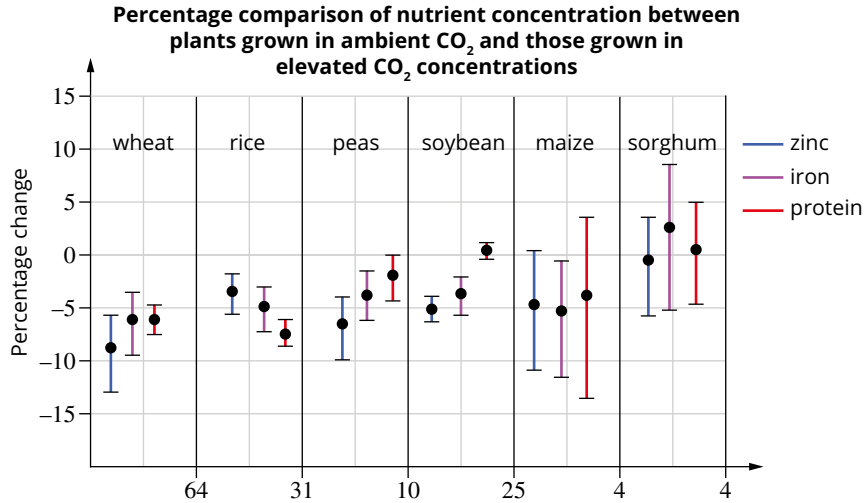


FIGURE 4.4.32 Percentage change in zinc, iron and protein in a number of crop plants when CO_2 concentrations are raised above the current ambient concentration. Error bars represent 95% confidence intervals and numbers along the bottom show the number of replicates pooled to provide the mean data displayed. Generally, a greater number of replicates results in a smaller error range.

Rising temperatures associated with increased CO_2 will also impact ecosystems. As climates warm, the geographical ranges for various species will shift. For example, species suited to the warmth of the tropics may increase in geographical range. If the soils of polar latitudes are able to support them, the ranges of temperate plants may shift towards the poles. There is, however, no guarantee that the poorer-quality soils that are normally found in polar regions will provide the nutrients required by temperate adapted plants. As ecosystems are always dynamic, succession of some form will occur.

As plant species move into new areas, the herbivores that rely on them for food will follow, as will the predators that eat the herbivores.

Reliability of ice-core data

Ice cores have been used as source of information about the atmospheric composition in the past. A cautionary note, however: the interpretation of ice-core data relies on the validity of three underlying assumptions:

- one layer of ice is formed each year
- diffusion of molecules such as CO_2 occurs at a constant rate
- each ice core is typical of the environment prevalent at the time of the ice deposition.

FIGURE 4.4.33 The distinctive layers formed as a result of the compaction of snow to form ice can be seen in this ice cliff in Antarctica.

Timescales for ice cores are established by counting the layers of deposition (Figure 4.4.33). Snow deposition and compaction into ice is seasonal and the resulting layers can be counted, with the number of layers representing the number of years. The major source of error in this method is that if snow deposition is less than 10 cm in a year, the layering becomes difficult to identify and the possibility of error increases. One factor that helps increase the reliability of ice-core dating is the presence of ash layers. These are thin but distinctive layers formed when ash produced during major volcanic eruptions settles on the ice (Figure 4.4.34). If the volcanic eruption was large enough, there will be evidence in rock layers across the world and these can be dated. Ash layers, if present, help to confirm timescales.

Diffusion is a natural and mechanical process that is experienced by all molecules. Measurement of past CO_2 levels by analysing trapped atmosphere in ice cores must take this into account. Like all molecular movement, diffusion is affected by temperature—higher temperatures cause faster diffusion. CO_2 diffusion rates are fairly well-established but, for accuracy of concentration estimates, temperatures at the time of deposition and for a period of time afterwards need to be established.

Only a limited number of ice cores are taken and analysed but as a result of variation in climate, and even microclimates, throughout the world, the assumption that these are an accurate representation of conditions everywhere may be inaccurate.

In all of these situations that introduce a level of uncertainty about the accuracy of the results, analysis of more ice cores obtained at a greater number of sites, along with averaging data over longer timescales, increases the reliability of the data and any conclusions drawn from it.

FIGURE 4.4.34 This section of an Antarctic ice core shows a distinctive layer of volcanic ash.

WS 3.2.6

Introduction of new species

Human activities transfer organisms into places where they previously did not exist. The introduction of these species sometimes provides challenges to the species that formed the initial ecosystem. Foreign species often have no predators and may be better competitors than the native species. They may also have other characteristics that harm native species. For example, the introduction of cane toads into Australia has had devastating effects on the abundance of some native species.

Impact of cane toads on native species

Populations of both the northern quoll and two large species of goanna were monitored as cane toads invaded their territory in northern Queensland. Population surveys have shown that, by the end of the 1990s, northern quolls were no longer present in many areas of northern Queensland.

In 2002, cane toads reached the Mary River Ranger Station in the southern part of Kakadu National Park. By March 2003, population surveys found no northern quolls. Examination of quoll bodies in the Mary River area showed at least 31% of them had signs of cane-toad poisoning.

The northern quoll eats a very varied diet of insects, frogs, small mammals, birds and reptiles. They are considered to be a keystone species due to their varied diet. They help to maintain the population balance between species in the tropical areas of northern Australia.

It is predicted that as cane toads spread further (Figure 4.4.35), the range of the northern quoll will be reduced by 95%. It is possible that they will become extinct on mainland Australia, and the only living members of the species will be small groups confined to islands off the Australian coast.

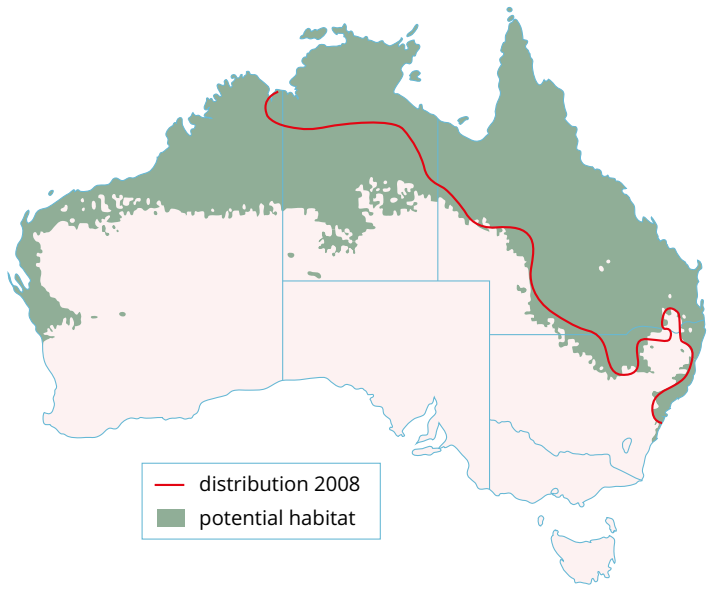


FIGURE 4.4.35 The possible future distribution of cane toads.

It is difficult to predict how the loss of the northern quoll will affect the ecosystems of northern Australia, but the quoll's role as a keystone species means that the effects are likely to be significant.

Large goanna species are also considered to be at risk. Following the arrival of cane toads in Kakadu National Park, the population of goannas declined by between 50% and 70%. Large goannas are one of the top predators of the ecosystems of northern Australia. Their loss is likely to result in a top-down trophic cascade (Module 4.3) leading to significant changes in the make-up of the communities of north Australia.

Predicting the impacts of human influences on ecosystems and their communities is difficult because ecosystems are complicated, highly interactive and always dynamic. For example, it was predicted that the arrival of the cane toads in the Adelaide floodplains of the Northern Territory would result in the reduction or local extinction of a number of snake species, but this has not eventuated.

The predictions and observed consequences on snake numbers after the introduction of cane toads are shown in Table 4.4.4. These results show how difficult it is to predict what will occur. Further studies suggested that the snakes were not impacted as expected because the cane toads caused major declines in the numbers of large lizards, which both competed with and fed on the snakes. Even though some snakes were being lost to poisoning by cane toads, overall numbers increased.

TABLE 4.4.4 Predicted and observed impacts of cane toads on numbers of various snake species on the Adelaide floodplains of the Northern Territory

Species	Predicted impact of toads	Observed impact of toads
Children's python	decline	increased
death adder	decline	increased
brown tree snake	decline	tended to increase
carpet python	decline	increased
small-eyed snake	decline	increased
water python	none	declined

Delving further into the ecology of the area showed that even the decline in water pythons was coincidental and not causal. The year prior to the study saw some major flooding. This reduced the numbers of rats, which were one of the main food sources of water pythons. Reduction in water python numbers therefore had more to do with a reduction in their major food source than the arrival of the cane toads.

This is an important lesson in how difficult it is to predict future responses of ecosystems to changes in the environment, whether they are biotic or abiotic. In the Adelaide floodplain, the results of change were less serious than predicted, but they could have been catastrophic, resulting in ecosystem collapse and the extinction of some species.

PA 3.2.3

Australia's Aboriginal and Torres Strait Islander peoples and ecosystems

Australia's Aboriginal and Torres Strait Islander peoples have an intimate knowledge of the land and the habits of our flora and fauna. Conservationists and ecologists are now working closely with Australia's Indigenous peoples in a number of areas to use that knowledge to preserve our natural heritage.

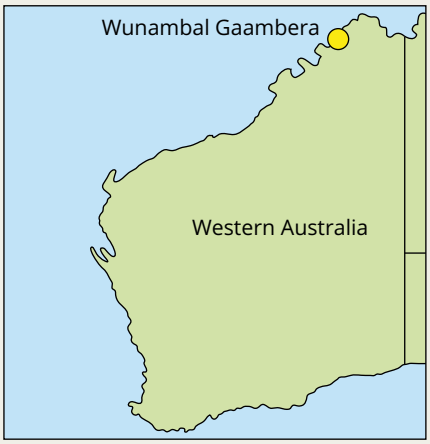


FIGURE 4.4.36 Location of Wunambal Gaambera country.

In the Wunambal Gaambera country in the Kimberley region of north-western Australia (Figure 4.4.36), ancient firestick farming practices are being used to reduce the intensity of bushfires and preserve native species of plants and animals. Each year, during the cool season, the rangers—Wunambal Gaambera traditional owners—set out on a five-day walk. During the walk, they set a mosaic of small cool fires that reduce leaf litter and encourage the growth of fire-requiring species.

These fires burn coolly, move slowly and extinguish themselves quickly. The slow-moving fires allow enough time for animals to escape into unburned areas and their small area means that food resources for the animals are not severely depleted. Because the fires are also cool, many plants survive the burn. While plants may lose their leaves, many Australian plants have epicormic buds that shoot immediately after a fire. Some plants even require their seeds to be burned before they can germinate. These cool fires allow enough burning for seed germination, unlike wildfires, which completely destroy seeds. Some animals rely on the sudden new growth after fire to support reproduction, so these regulated burns help maintain the ecosystem.

The Wunambal Gaambera traditional owners renewed 'right-way fire burning' when they regained title to their land in 2011. Since then, they have established the Uunguu Indigenous Protected Area, and the number of serious summer bushfires has decreased. In addition, the populations of endangered species of plants and animals, like the northern quoll (Figure 4.4.37), have flourished.

FIGURE 4.4.37 The endangered northern quoll.

The burning practices of the Wunambal Gaambera people have informed researchers and ecological experts about how to reduce the chances of devastating fires in the future. These practices also offer insight into the management of Australian parks and bushland to help protect many endangered species and maintain the biodiversity of our continent.

It is not only on the land that Indigenous understanding of the Australian environment is informing scientific practices. Both Aboriginal and Torres Strait Islander peoples have hunted dugongs for food for thousands of years. Their accumulated understanding of dugong behaviour has allowed scientists to build up a clear picture of the numbers and movements of this now-endangered species. Dugongs are gentle creatures that feed on seagrass beds. Their natural range is broad (Figure 4.4.38). They were once found in the waters of at least 37 countries but the loss of many of the seagrass beds throughout the world, mostly as a result of human activities, has severely impacted on their numbers.

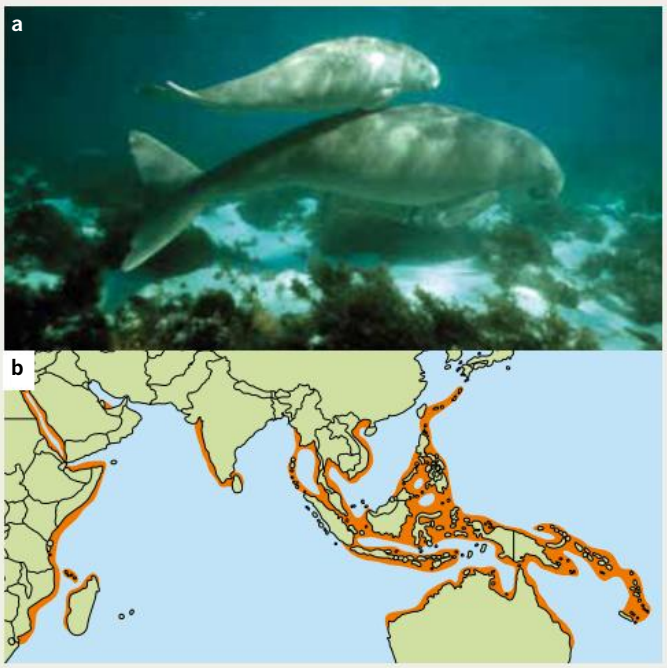


FIGURE 4.4.38 (a) Dugong mother and calf. (b) The natural range of the dugong.

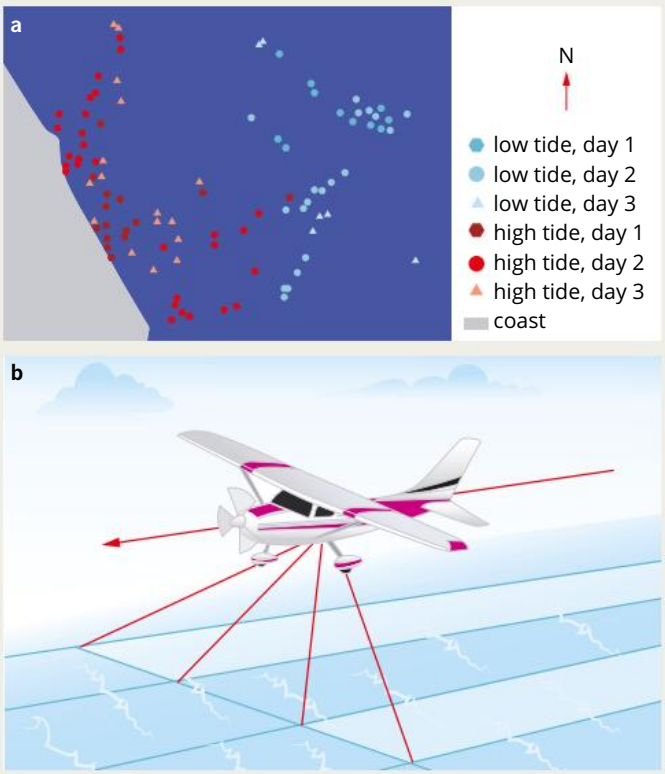


FIGURE 4.4.39 (a) Satellite tracking data of tagged dugongs off the Queensland coast, showing their movement according to the state of the tides. (b) Dugong aerial surveys are conducted along defined tracts by planes flying at constant heights and speeds.

Adaptation of Indigenous hunting techniques has allowed scientists to tag and monitor dugong. Tagging a marine creature can be a challenge. In some areas, Indigenous hunters are assisting scientists by affixing the tags to the dugongs. The tagging process involves chasing a dugong in a small boat until it tires, then diving into the water and gently wrestling the dugong so that the transmitter can be attached to it. This process takes some skill if the dugong is not to be harmed. This is especially difficult because dugongs are very timid creatures that dive deeply when frightened.

The dugong habit of diving when frightened can also make obtaining population estimates very difficult. Indigenous people also noted that dugongs have particular feeding habits. Dugongs return to the same seagrass bed on successive tides for substantial periods of time before they move elsewhere. However, they always eventually return to their favoured feeding grounds. This knowledge about where dugongs are likely to be encountered and how they react to noise is vital for making accurate observations of the dugong population size. Counts can be wildly inaccurate without this knowledge. Population numbers can appear very healthy if their favoured feeding ground is surveyed, but a subsequent survey may suggest a population crash if the survey does not include the favoured seagrass beds. Also, if the timidity of the dugong is not considered, underestimates of numbers will occur. It is impossible to count a dugong that has been frightened and has dived. One way to make accurate counts is to use aerial surveying techniques that take into account the behaviour of the dugong. Another way is through tagging and satellite tracking (Figure 4.4.39).

Review

1. Explain why the 'right-way fire' burning of the Australian landscape is more ecologically sound than stopping fires whenever possible.
2. Explain why both aerial surveys and tagging with transmitters are needed to monitor dugongs.
3. Discuss how Indigenous knowledge of the land helps to conserve Australian flora and fauna.

4.4 Review

SUMMARY

- Ecosystems are dynamic and change over time. Many changes in the environment are rhythmic.
- Growth and reproduction of organisms, abundance of food in food chains and rates of cycling of nutrients also change seasonally.
- Ecosystems gradually change in an orderly fashion through the process of succession. During succession, biological communities replace one another in sequence over time. Each successive community is a sere.
- The causes of succession are both physical processes in the environment and the effects of organisms interacting with one another and their surroundings.
- Primary succession begins with the colonisation of a bare, previously uninhabited area.
- Secondary succession follows disturbance of an existing biological community. Human activities are often the cause of such a disturbance.
- The first organisms in a succession have traits that allow them to survive in harsh conditions. These are called pioneers and they form a pioneer community. Pioneer communities are generally simple in their structure.
- The last sere in a succession is the climax community. Climax communities have a complex structure.
- Eutrophication is a process whereby organic material builds up in a water source to such an extent that the decomposition of organic material uses all of the oxygen and creates an anoxic environment.
- Eutrophication is a final stage in the succession of a lake to dry land.
- Fossils can be used to identify changes in the past environment.
- Ice cores give information about atmospheric composition and rock cores contain fossils of plants and animals.
- Humans affect living organisms by hunting them, introducing non-native species and planting monocultures, which reduce biodiversity. Because organisms interact with one another, human activities influence food webs.
- Humans clear land, which causes habitat loss and habitat fragmentation and leads to reductions in carrying capacity and confining organisms to islands of habitat where inbreeding and loss of genetic diversity may occur.
- Humans burn fossil fuels, which increases atmospheric carbon dioxide levels that may be harmful to plant productivity and contribute to global temperature increases.
- Dryland salinity occurs when water tables rise, because deep-rooted plants that normally take up water have been removed during land clearing.

KEY QUESTIONS

Retrieval

1 Define climax community.

2 **a** Recall what is meant by pioneer species.
b List three characteristics that make a species a successful pioneer.

3 Describe microfossils.

Comprehension

4 A farmer cleared some land and planted wheat crops. Following five years of good wheat production, she discovered that her crop was dying and a white crust of salt was forming in low lying areas of her land. Explain whether the planted wheat crop would be considered a primary or secondary community.

5 Explain why non-native species often become pests when they are introduced into new environments. Refer to interspecies relationships.

Analysis

6 Roadside vegetation in semirural areas often consists of three zones. The zone nearest the road is mowed regularly and consists of herbs and grasses. The next zone away from the road is not mowed, but is sprayed once a year with herbicide to kill weeds and young tree seedlings. This second zone consists of shrubs and weeds. The third zone, furthest from the road, is unmanaged and is open eucalypt forest with many weeds.

a Explain why the first zone mainly features grasses rather than shrubs.

b Deduce what changes you would you expect in both the grass and shrub zones if herbicide spraying and mowing were both stopped. Explain your reasoning.

7 Figure 4.4.27 on page 208 shows the relationship between population size and heterozygosity.

a Describe the relationship between population size and heterozygosity.

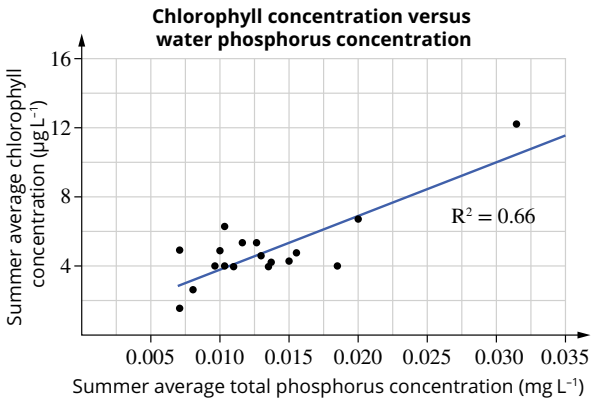
b Infer what this graph indicates about the size of population needed for high levels of genetic diversity.

c Evaluate how the data is displayed in this graph and the effect this has on how it is interpreted.

8 One way to measure the increase in phytoplankton in a waterway is to measure the concentration of chlorophyll.

a Discuss why the concentration of chlorophyll may be used as an indicative measure of phytoplankton.

b Consider the graph below. Describe what you can infer.



c Deduce, with justification, the relationship between phosphorus concentration and chlorophyll concentration.

d Explain the link that scientists hypothesise between common blooms of toxic cyanobacteria in Australian waterways during periods of drought and the increasing use of fertilisers, such as superphosphate, by farmers.

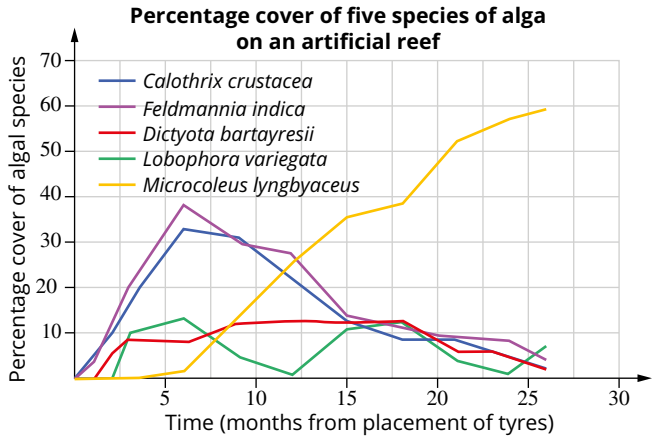
9 A student drilled a core from an area 1 km from the coast in a eucalypt rainforest and found the following fossil layer in the core.

layer A	eucalypt pollen, fern spores
layer B	eucalypt pollen, grass pollen
layer C	grass pollens, pigface pollen
layer D	mangrove pollen
layer E	clam and mussel shells
layer F	grass pollens, pigface pollen
layer G	mangrove pollen
layer H	clam and mussel shells

a Deduce, with justification, how the ecosystem has changed over time.

b Identify the oldest layer.

10 A series of tractor tyres were sunk into a lagoon in the tropics to create an artificial reef. Over a period of 26 months, the growth of algae on the tyres was monitored. By 30 months, only *Microcoleus lyngbyaceus* and *Lobophora variegata* were evident on the tyres. The graph below shows the results of the monitoring.



a i Identify the species that could be considered to be pioneers.

ii Identify the species that is/are showing a seasonal pattern of abundance.

iii Name the species that belong to a climax community.

b One year after the monitoring ceased, a ship discharged its ballast in the lagoon and a herbivorous sea star was released.

i Recall the term used to describe the sea star.

ii Predict what would happen to the population of *Feldmannia indica* over the following year if the sea star preferentially fed on *Microcoleus lyngbyaceus*.

MP 3 | SAT IA2

Comparing plant diversity between two ecologically similar sites

Research and planning

Aim

- To compare plant diversity between two ecologically similar sites
- To investigate how quadrats can be used to survey a population
- To evaluate the use of random quadrat sampling as an ecological surveying technique

Note: Another ecological surveying technique could be used instead of quadrat sampling. Other techniques include transects, point sampling and aquatic sampling techniques.

Rationale (scientific background to the experiment)

A population is defined as members of the same species living in the same geographical area at the same point in time. In other words, they have the ability to interbreed to produce viable offspring. It is necessary to survey populations for many reasons. For example, a population may be endangered or increasing in numbers and impacting negatively on another species. Information about population sizes can assist in forming decisions in regards to land management and conservation efforts.

For terrestrial ecosystems, quadrat sampling is a useful method for assessing the abundance of plants or slow-moving animals within a given area. However, depending on the survey area, it can be a labour-intensive method of sampling. Transects may include line or belt transects and can be used in conjunction with quadrat sampling. Transects are particularly useful for assessing the changes in a community along an environmental gradient such as the intertidal zone or a mountainous incline.

To investigate aquatic ecosystems, a range of sampling techniques can be used, including netting and sediment samples. The methods used to survey aquatic populations depend on several factors, including the type of organisms being analysed, the speed of water flow and depth of water.

Timing

60 minutes

Materials

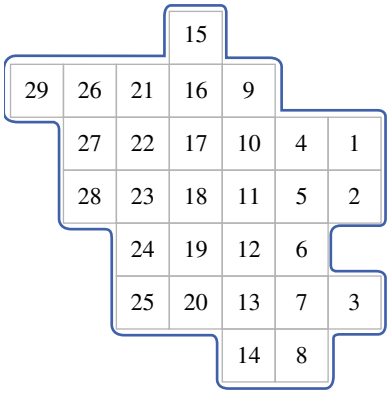
- 1 m^2 quadrat (larger quadrats can be used for trees and shrubs)
- two ecologically similar sites
- street directory or internet
- numbered grid
- random number generator
- device to measure total surface area of land being surveyed (e.g. trundle wheel, tape measure or laser tape measure)
- field guide to select species for sampling (e.g. guide to common weeds)

Method

Risk assessment

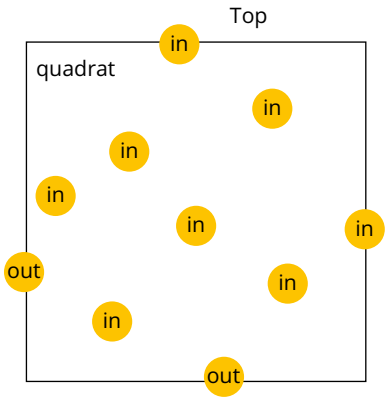
Assessment of risks include chemical hazards and physical hazards. Before you commence this practical activity, you must conduct a risk assessment. Complete the template in your *Pearson Biology 12 Queensland Skills and Assessment* book or download it from your eBook.

1. Consider the local environment surrounding your school and select two ecologically similar sites to survey. If you choose areas of grass, it is better to choose areas that have not been mown recently so that the weeds are clearly visible among the grass species.
2. It might be useful to use a field guide to become familiar with local flora.
3. Obtain maps of the two sites, either from a street directory or the internet. Estimate the total surface area of the sites to be surveyed. This will be more challenging for an irregular shape. Record the shape and approximate dimensions.
4. Lay a numbered grid over the maps. Alternatively, you may use a ruler to grid the maps yourself.



5. Use a random number generator to select five sampling sites for each location. Note: If there is more time available, you can increase the number of sampling sites.
6. When you have confirmed the locations of the sampling sites, you can begin your population surveys.

7 At your first sampling site, place the quadrat on the ground and systematically count and record the type and number of plant species observed. This will give you a number per m^2. Only count plants that are partially inside the quadrat if they cross the top or right side. Exclude plants that cross the bottom and left side of the quadrat.



8 Continue to repeat step 7 for all of your quadrat samples.

9 Calculate an average number of plants per m^2.

10 Scale this up to estimate the total number of plants within the survey area.

11 Repeat steps 7–10 for the second site.

12 Compare the information collected to those in other groups.

Analysing

Raw data

1 Record your data in a table similar to the one below.

Site 1

Species	Quadrat sample					Average
	1	2	3	4	5	
Total						

Total number of species:

Site 2

Species	Quadrat sample					Average
	1	2	3	4	5	
Total						

Total number of species:

Processed data

2 From the raw data, calculate the species richness at each location.

3 Use a spreadsheet to calculate Simpson's diversity index for each site.

When using multiple quadrats to sample a site, Simpson's diversity index is calculated using the following formula:

$$\text{SDI} = 1 - \left(\frac{\Sigma n(n-1)}{N(N-1)}\right)$$

where

n = average number of organisms of one species

N = average total number of organisms of all species.

Alternatively, you could use a table like the one below to manually calculate Simpson's diversity index.

Site 1

Species	n	$n - 1$	$n(n - 1)$
A			
B			
C			
D			
E			
Total	N =		$\sum n(n-1)$ =

➤ Reflect and check that your data analysis demonstrates these characteristics

- ☐ Effective investigation of phenomena is demonstrated by the collection of sufficient and relevant raw data
- ☐ Accurate application of algorithms, visual and graphical representations of data is demonstrated by appropriate processing and presentation of data to aid the analysis and interpretation of data

Analysis

4 Explain the difference between species richness and Simpson's diversity index.

5 Identify the site that had the most biodiversity.

6 Use Simpson's diversity index to identify which community had the least biodiversity. Explain why the Simpson's diversity index was low for this community.

7 Identify three benefits of quadrat sampling.

8 List any difficulties or challenges you had with your ecological surveying technique.

9 Explain whether you could have used the same sampling technique to compare species diversity of birds in the same area.

10 Consider whether another sampling technique would have been more appropriate for the two sites you sampled. Explain why or why not.

➤ Reflect and check that your analysis demonstrates these characteristics

- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough and appropriate error analysis
- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough identification of relevant trends, patterns and relationships
- ☐ Insightful and valid interpretation of evidence is demonstrated by drawing a valid and defensible conclusion based on the analysis

Interpreting and communicating

Conclusion

1 Summarise the advantages and disadvantages of using quadrats as an ecological surveying technique.

Improvements

2 If you were to repeat this experiment, identify the steps that you would do differently. Consider:

- **a** how you might change the methodology
- **b** how you might improve your technique
- **c** how you could reduce error and uncertainty.

Extension

3 Studies of biodiversity are an important component of assessing environmental health as well as the impact human activities have on environments. You have been asked to determine the environmental impact a chemical factory is having on a nearby river. Explain how you would go about sampling the local area to determine the factory's environmental impact.

4 Use a different sampling technique to calculate the species diversity of the two sites you have just analysed. Compare the measurements for species diversity of the two sites. Explain which sampling technique was the most appropriate.

➤ Reflect and check that your evaluation demonstrates these characteristics

- ☐ Critical evaluation of processes is demonstrated by a discussion of the reliability and validity of the experimental process supported by evidence such as the quality of the data (as quantified in the error analysis)
- ☐ Critical evaluation of the conclusion is demonstrated by a discussion of the veracity of the conclusions with respect to the error analysis and limitations or sufficiency of the data
- ☐ Insightful evaluation of processes and conclusions is demonstrated by a suggestion of improvements or extensions to the experiment that are logically derived from the analysis of the evidence

Chapter review

WS 3.2.7 WS 3.2.8

04

KEY TERMS

adenosine triphosphate (ATP)
algal bloom
allelopathy
amensalism
ammonification
anoxic
atmosphere
autotroph
biogeochemical cycle
biomass
biome
biosphere
carbon fixation
carnivore
carrying capacity
chemoautotroph
chemosynthesis
chemosynthetic autotroph
climax community
commensalism
community
competitive exclusion
decomposer
denitrification
denitrifying bacteria
density-dependent factor
density-independent factor
detritivore
detritus
ecological niche
ecological pyramid of energy
ectoparasite
emigration
endoparasite
environment
equilibrium
eutrophication
exponential growth
food chain
food web
fundamental niche
gross primary productivity
habitat
herbivore
heterotroph
host
hydrosphere
immigration
interspecific competition
intraspecific competition
keystone species
limiting factor
Lincoln index
lithosphere
logistic growth
mesopredator
migration
mortality
mutualism
natality
net primary productivity
niche
niche partitioning
nitrification
nitrifying bacteria
nitrogen-fixing bacteria
omnivore
parasite
parasitism
photoautotroph
photosynthesis
photosynthetic autotroph
pioneer community
pollinator
population
population crash
population explosion
predation
predator
primary productivity
primary succession
realised niche
saprophytic bacteria
scavenger
secondary succession
sere
succession
symbiont
symbiosis
trophic cascade
trophic level
vector
zonation

KEY QUESTIONS

Retrieval

1 Select the process carried out by bacteria in the soil that converts nitrates into gaseous nitrogen, which is then released into the atmosphere.

A nitrification
B denitrification
C decomposition
D ammonification

2 Define niche.

3 Define biomass.

Comprehension

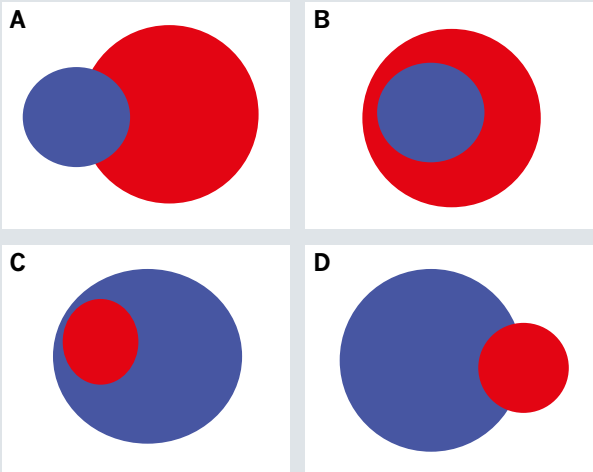
4 Nuthatches and brown tree creepers are both birds that feed on insects in trees. The nuthatches feed mostly in the canopy while the brown tree creepers feed primarily on the trunk. Select the cause of these behaviours.

A niche partitioning
B a trophic cascade
C competitive exclusion
D they are both keystone species

5 Select which of the following situations describes a population that is increasing in size.
B = birth, D = death, I = immigration, E = emigration

A $(B + D) > (I + E)$
B $(B + E) > (D + I)$
C $(B + E) < (D + I)$
D $(D + E) < (B + I)$

6 Select which of the following diagrams most accurately illustrates the relationship between the fundamental and realised niches of a species. Red circles represent the fundamental niche and blue circles represent the realised niche.



7 Select the statement below that explains why introduced species frequently demonstrate the principle of competitive exclusion.

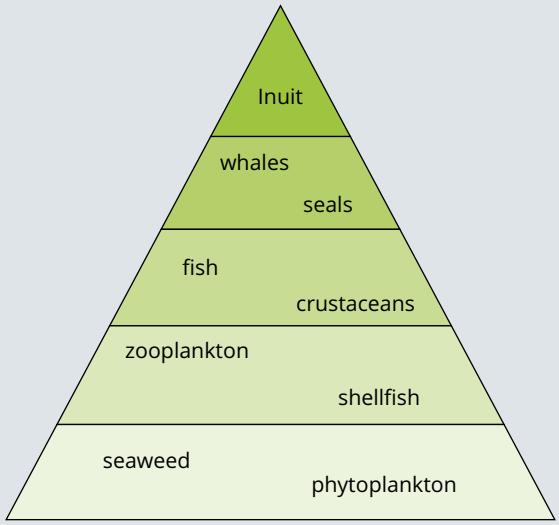
A Introduced species are able to reproduce at a higher rate than native species.
B Native species are more efficient at utilising natural resources than introduced species.
C Introduced species occupy the same ecological niche as native species and compete for resources.
D Introduced species are not held in check by the predators and diseases that affect native species.

8 Select the change in abiotic factor that would have the greatest negative impact on the carrying capacity of a billabong ecosystem.

A increase in turbidity by 10%
B decrease in nutrients by 10%
C increase in water level by 10%
D decrease in wind speed by 10%

9 Explain the difference between primary and secondary succession.

10 Consider the following diagram, which represents a marine pyramid of biomass.



a Select the level in the pyramid that represents:
  i the greatest amount of energy
  ii the least amount of energy

b Only a small proportion of the energy that passes from level to level along food chains reaches the top carnivores. Describe what happens to the rest of the energy.

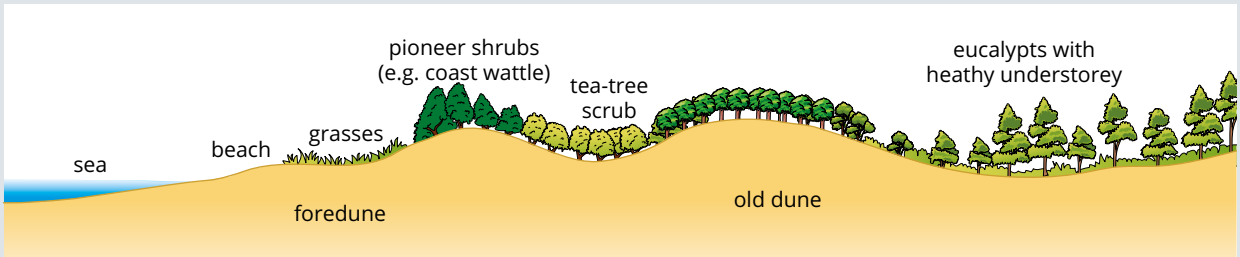
11 Coastal sand dunes are an example of succession. The diagram below illustrates the changes in vegetation on the coastline.

The sand of the foredune (closest to the water) is colonised by highly specialised plants, including grasses such as the native hairy spinifex and the introduced marram grass. The old dune is the hill before the eucalypts.

a Describe the characteristics of grasses that enable them to colonise the foredune.

b Describe the environmental changes from the foredune to the old dune. Consider biotic and abiotic factors.

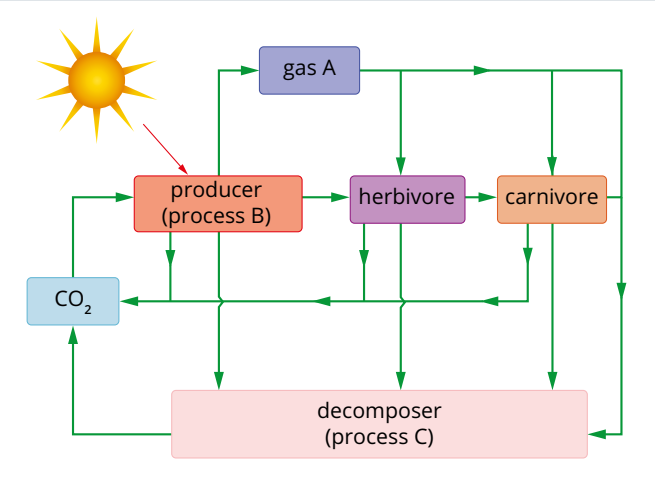
c Explain which environment is more stable: the foredune or the old dune.



12 Baleen whales, including the blue whale, are the largest mammals in the world. They have rows of large, comb-like baleen plates instead of teeth. They feed on small crustaceans (such as krill), shellfish and plankton, which they strain from the water through the baleen plates. Discuss the benefit of this diet for such a large animal. Make reference to trophic levels.

13 Explain the advantage of planting clover in a lawn.

14 Consider the diagram below, which shows the biogeochemical cycle of one particular element.



a Name the element being shown as cycling in this system.

b Identify:

i gas A

ii process B

iii process C

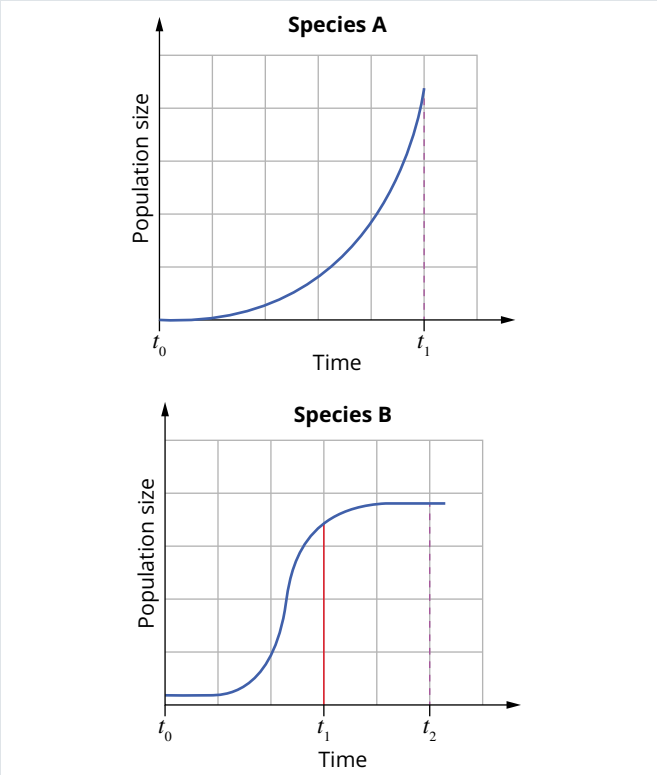
c Summarise the role that decomposers play in ecosystems.

d Explain how detritivores and decomposers are different.

15 Describe the relationship between a habitat and a niche.

Analysis

16 The two graphs below show the population growth of two species, A and B.



Deduce which of the following statements correctly identifies the situation demonstrated by the graphs.

A No deaths occurred in either species between times t_0 and t_2.

B Species B has reached the carrying capacity of the ecosystem by time t_2.

C The graph for species B is typical of an insect population such as a locust plague.

D Species A shows an exponential population growth between times t_0 and t_1 but species B does not.

17 A group of koalas was surveyed for several years.
Number of births, number of deaths and migration were all monitored.
The data is shown in Tables A to C.

Table A: Annual births 1985 to 1994

Year	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
number of births	12	16	11	15	6	9	12	19	22	7

Table B: Annual deaths 1985 to 1994

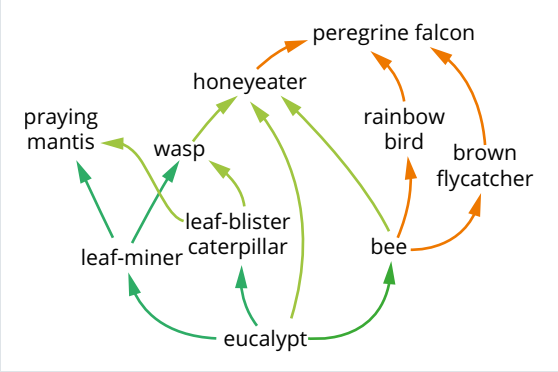
Year	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
number of deaths	4	9	3	8	12	15	7	6	12	5

Table C: Migration 1985 to 1994

Year	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
immigration	3	6	3	5	1	2	4	5	5	2
emigration	5	2	4	3	7	1	6	2	2	7

Calculate the rate of population change for the first five years of the study and compare it with the second five years of the study.

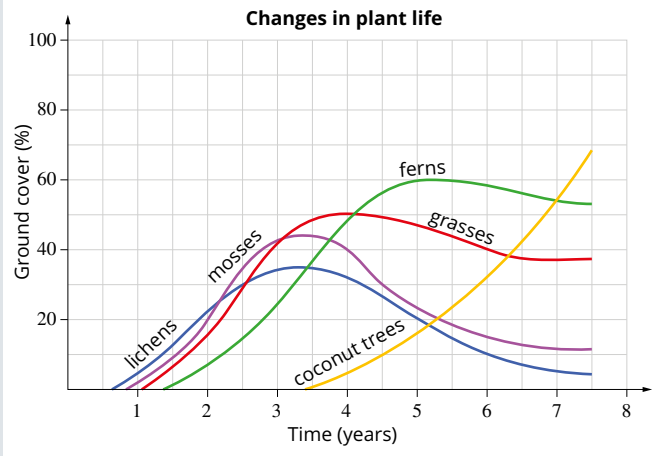
18 The bee population of a woodland community, illustrated by the food web shown below, is under threat from infection by the varroa mite. The varroa mite is a parasite that infects bees. Ecologists expect that the invasion of the mite will result in the loss of the entire bee population.



a Predict the probable effects on the populations of the following members of the food web resulting from the loss of the bees. Justify your predictions. If the effects cannot be determined, explain why.
- **i** rainbow bird
- **ii** peregrine falcon
- **iii** wasp
- **iv** leaf-miner

b Predict the long-term consequences for the ecosystem following the extinction of the bees.

19 The graph below illustrates the changes in plant life observed over several years on one of the islands previously known as Krakatau from 1890. A volcanic eruption in 1883 split the island into two, eradicating all animal and plant life across both new islands. In 1890, scientists noted that animals such as insects, spiders, snakes and lizards were present on one of the islands. A thriving forest community still exists today.



a **i** Draw a flow chart showing the order of successive plant types growing in the years following the eruption.
- **ii** Annotate your flow chart to clearly discuss the possible role of each plant type in the establishment of subsequent plant species.

b Infer how the following organisms may have reached the island after the volcanic event:
  i mosses, ferns and grasses
  ii insects and larger animals

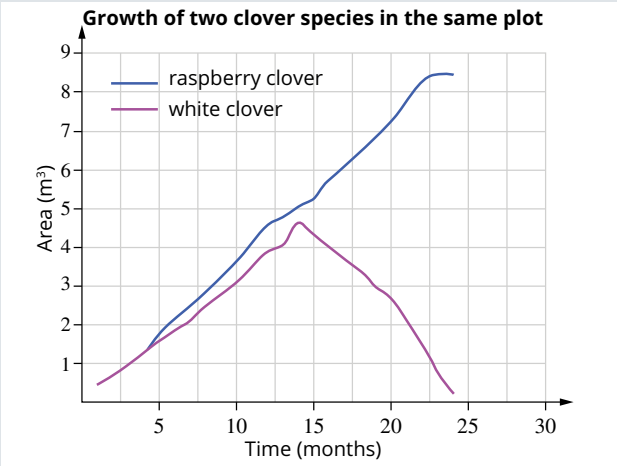
20 In a study examining rice (*Oryza sativa*) growth response to salinity, the following results were obtained.

Oryza sativa growth response to salinity

Treatment/ salinity	Days to maturity	Fertility (%)	Weight of 1000 seeds (g)
2 dS m^{-1}	143.1	90.58	25.63
6 dS m^{-1}	137.1	85.09	22.14
8 dS m^{-1}	135.1	76.92	20.73
12 dS m^{-1}	134.8	52.49	16.33

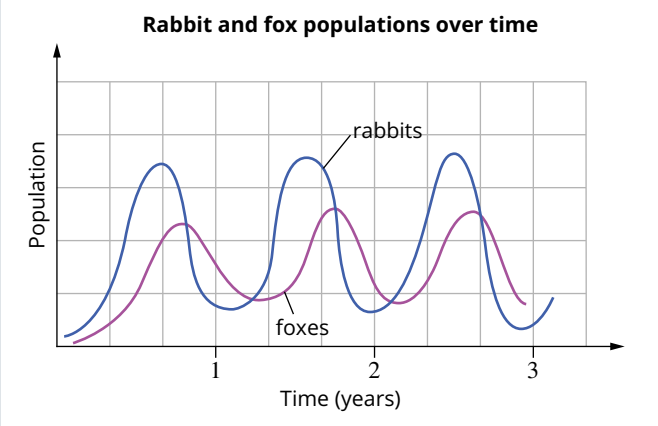
a Describe how salinity influences the life cycle of rice plants.
b Explain the effect of increasing salinity on rice yield.
c **i** Draw a graph of salinity versus fertility for rice.
  ii Deduce how salinity influences plant fertility. Use the graph.
d In several areas of Australia, including the Murray–Darling basin, irrigation has resulted in a rise in the water table. The rising water table has caused salt from deep underground to rise, contaminating surface soils. Kangaroos are a major mammalian herbivore of the Murray–Darling basin. Infer how this salting will affect the carrying capacity for kangaroos in these areas.

21 Two species of clover are often planted by farmers to increase nitrates in their fields: white clover and raspberry clover. The graph below demonstrates their growth when planted in the same field.



Explore the relationship between raspberry clover and white clover.

22 Consider the following graph, which shows the rise and fall of rabbit and fox populations on an isolated area of open farmland in central Queensland.



a Determine factors that might have caused the initial growth in the rabbit population.
b Propose a limiting factor of the fox population.
c Determine factors that could cause the decline in the rabbit population.
d Explain how rabbit numbers are able to build up again in the year following their decline.
e Extend the graph to illustrate a crash in the rabbit population.

23 A farmer excavated a large dam on his property to supply water to his sheep. He enjoyed eating yabbies, so he obtained six yabbies from a neighbour's dam and put them in his own. After allowing them to breed for six months, his daughter Sara, who was studying biology, decided to find out how many yabbies were now in the dam.
a Sara placed a yabby trap in the dam and collected 12 yabbies. She marked them and then released them back into the dam. The next day she replaced her trap and captured 10 yabbies, 6 of which were marked. Calculate the population of yabbies in the dam.

Over the next four years, Sara continued her experiment and collected the following data by sampling the dam every 6 months:

Yabby population

Months since yabbies were introduced into the dam	Population size
12	40
18	80
24	120
30	160
36	155
42	160
48	160

b Graph the data appropriately.

c Identify what kind of growth is being shown by the yabby population.

d Infer a cause for the population measurement at the 36th month of the study.

24 A study of energy transfer in a particular ecosystem produced the following information. All energy is measured in kJ m^{-2} y^{-1}.

Ecosystem energy transfer

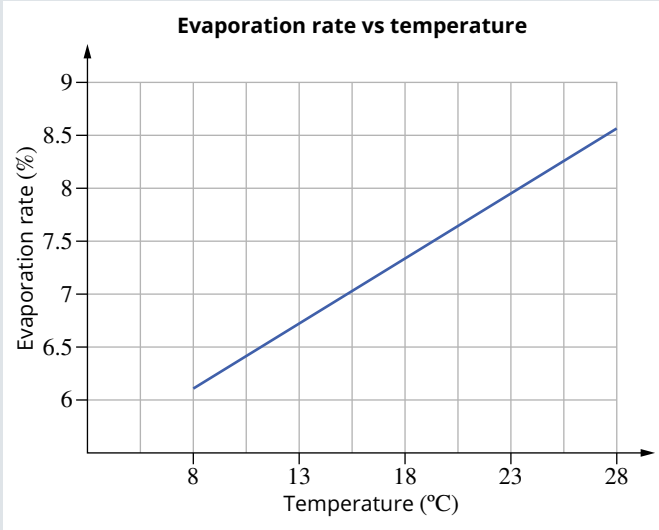
Energy production or removal	Producers	Primary consumers	Secondary consumers
converted to maintained biomass	30 160	3030	560
converted to biomass but eaten by consumers	7340	1470	0
metabolism	8760	1920	750
total production	46 260	6420	1310

a Calculate the percentage of the total energy captured by the producers that is transferred to the primary consumers. Show your working out.

b Calculate the percentage of the total energy captured by the producers that is transferred to the secondary consumers. Show your working out.

c Assuming the solar energy falling on the ecosystem is 2 190 000 kJ m^{-2} y^{-1}, calculate the percentage of solar energy falling on the ecosystem that is not incorporated into biomass.

25 A study of the effect of temperature on evaporation rates in Australian waterways was undertaken. The results are shown in the graph below.



a Use the graph to determine the increase in evaporation rate due to a 2°C rise in temperature.

b Scientists have predicted a temperature rise of between 1.7°C and 5.1°C over the next century due to increases in the CO_2 concentration in the atmosphere. Predict how these temperature increases will affect eutrophication of Australian waterways. Explain your reasoning.

26 Consider the fossil sequence shown in the pictures on the next page, which is the result of a core drill at a single site.

a Scientists draw inferences about past environments through observing the fossils present in a layer of rock formed in ancient times. Draw inferences about the environment present at the time of deposition of each layer.

b Dating placed the age of layer E at 400 million years old and layer A at 25 million years old. A student examining the core drill stated the sequence demonstrated a timeline of ecological change in the area through the period between 500 and 25 million years ago. Discuss the validity of this statement.

A

B

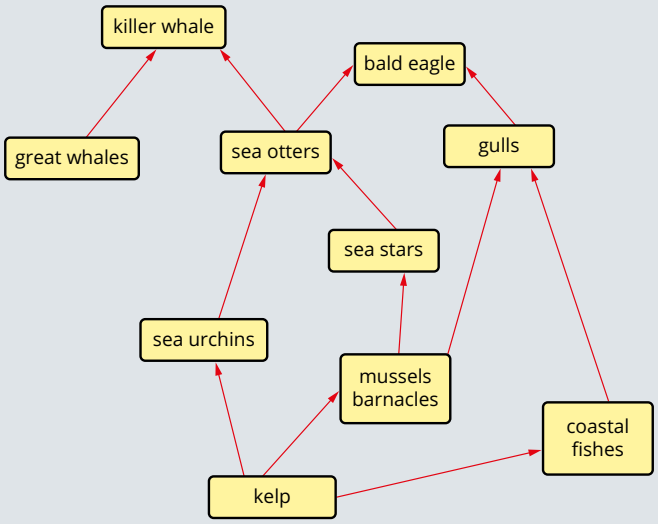
C

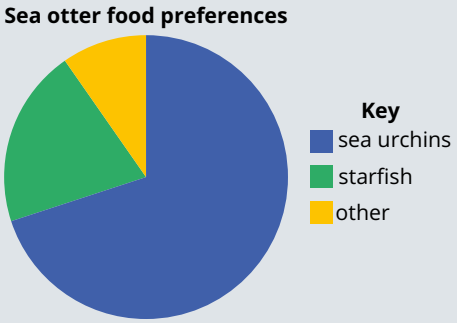
D

E

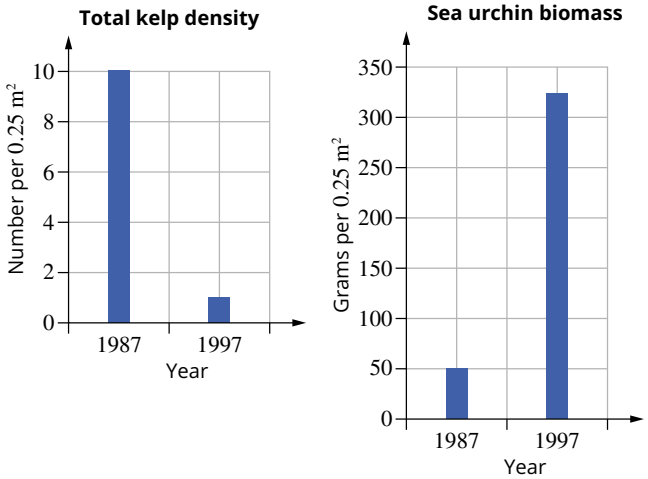
Knowledge utilisation

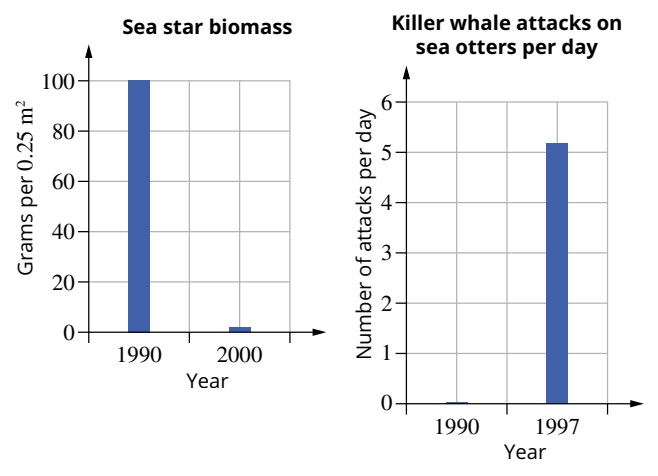
27 The fisheries off the coast of Alaska have undergone a severe decline in recent years. A simplified version of the food web associated with the coastal fisheries, showing the major organisms involved, is shown below.

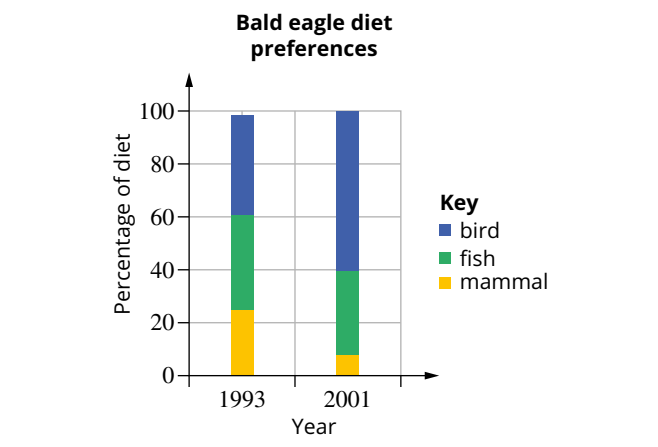


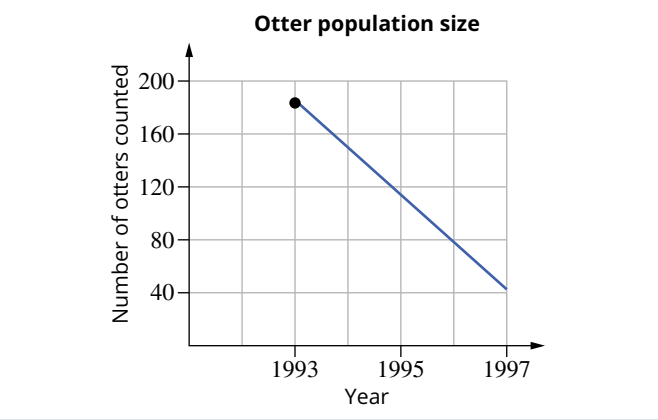


Studies were undertaken to identify the cause of the collapse. Data on population sizes before, during and after the collapse, along with some other data collected, are provided in the following graphs and tables.









a Explain why, after the decline in sea otter numbers, the sea stars also declined.

b Ecologists have identified the sea otter as a keystone species in this ecosystem. Using the data provided, explain how this decision was made.

c Propose how the area might be monitored in an experiment aimed at determining if the kelp beds could recover if all sea urchins were removed and the area was netted to keep them out.

d Devise a research question to investigate why the coastal fisheries have collapsed.

e i Prior to 1990, no killer whale had been seen to attack a sea otter. Ecologists have postulated that this new behaviour has been caused by human activities and is related to the reduction in other food supplies used by killer whales. Calculate how many sea otters each killer whale consumes per year. Use the data to help.

ii Prior to 1990, the population of sea otters at one particular site was estimated to be 52 700. Over the next six years, the population decreased by 75%. Calculate the number of killer whales that must have started feeding on the sea otters to cause this rate of decline.

iii Discuss why your estimate of the number of killer whales involved is likely to be an underestimate. Decide what further information you need to make a more accurate estimate of the number of killer whales involved.

28 a Researchers have concluded that a trophic cascade is occurring in the southern waters off the coast of the USA, as discussed on pages 166 to 167. Predict how the populations of mesopredators should change after the year 2000.

b Local scallop fishers in the study area particularly blamed the rise in cownose rays for the decline in scallop numbers. Discuss whether the data supports their view. Consider the data shown in Figure 4.2.22 (page 167) when formulating your answer.

c During a recent critical scientific review of the 2002 study, the thermal tolerances of some of the species involved were investigated. The results are shown in the table below.

Thermal tolerances of mesopredators and apex predators

		Temperatures (°C)		
	Species	**Minimum**	**Normal range**	**Maximum**
mesopredators	chain catshark	8	8–12	12
	little skate	2	3–14	20
	cownose ray	15	16–22	30
apex predators	dusky shark	11	17–30	30
	tiger shark	11	17–30	30
	blacktip shark	18	18–33	33
	bull shark	21	21–33	33
	sandbar shark	14	17–28	28
	scalloped hammerhead	18	18–27	27

Discuss whether this data supports the contention that the changes in population size of the mesopredators, illustrated in Figure 4.2.22, are the result of a trophic cascade due to the loss of apex predators.

d One criticism of the 2002 study was that the data on the apex predator numbers was based on a small number of individuals sampled at only two sites. Discuss the validity of this criticism.

e i Suggest any further data which could be collected in order to support or disprove whether the population crash in the scallops was due to a trophic cascade.

ii Assuming that, after gathering more data, it is shown that the population crash of the scallops had causes other than a trophic cascade. Explain if this disproves the entire concept of trophic cascades.

29 On the island of Surtsey, study quadrats have been established and monitored for changes in plant species and their abundance for several decades. One quadrat is situated on the lava flows within the area inhabited by the seagulls. The results of that monitoring for the period 1994–2008 are shown in the table below.

Changes in plant species and plant abundance, 1994–2008

	Year							
Species	**1994**	**1996**	**1998**	**2000**	**2002**	**2004**	**2006**	**2008**
Poa pratensis	0	0	0	1	2	2	1	2
Cochlearia officinalis	0	0	10	16	5	1	0	0
Festuca richardsonii	1	3	20	50	75	96	100	100
Stellaria media	0	3	2	1	0	0	0	0
Cerastium fontanum	0	1	7	13	6	1	0	0
Poa annua	0	0	1	1	2	2	1	2
Puccinellia distans	28	32	50	15	3	0	0	0
mosses	1	2	2	2	2	2	2	2

a Identify which of the species could be considered to be the most effective pioneer.

b Discuss how this data helps answer the research question, 'Is succession occurring on Surtsey?'.

c Another study quadrat has been established on similar terrain but at a considerable distance from the seagull colony. Propose what vegetation profile could be expected in the area. Justify your proposal.

UNIT 3 • REVIEW

REVIEW QUESTIONS

Topic 1 Describing biodiversity

TR 3.1 TR 3.2

Multiple choice

1 Identify the difference between a variable and a control in a biology experiment.

A Variables are always changing.
B All variables must be kept constant so that the experiment is a valid and controlled investigation.
C Variables can change in an experiment, and controls are used to ensure they don't change too much.
D Variables are the factors that could change in an investigation, whereas the control is a standard against which results can be compared.

2 Four species were under investigation: *Bubas bison, Bubas bubalus, Bison bonasus* and *Bubalus mindorensis*. Identify the two most closely related species.

A *Bison bonasus* and *Bubas bison*
B *Bubas bubalus* and *Bubas bison*
C *Bison bonasus* and *Bubalus mindorensis*
D *Bubalus mindorensis* and *Bubas bubalus*

3 Name the ecosystem in which biodiversity would be expected to be highest.

A desert
B grassland
C rainforest
D sclerophyll forest

4 The table below shows the percentage difference in DNA between four primate species.

Percentage difference in DNA in selected primates

Species	Chimpanzee	Gorilla	Orangutan
gorilla	2.3		
orangutan	3.6	3.5	
gibbon	4.8	4.7	4.9

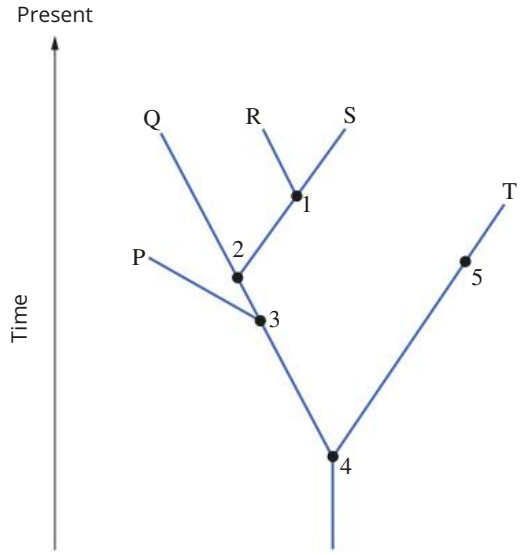
Determine which of the primate pairs shown below have the most distant relationship.

A gorilla and gibbon
B gibbon and orangutan
C chimpanzee and gorilla
D chimpanzee and orangutan

5 Identify the correct sequence for the classification hierarchy, from the broadest to the most specific grouping.

A species, genus, family, class, phylum
B phylum, kingdom, family, class, domain
C domain, kingdom, phylum, class, family
D species, family, order, kingdom, domain

Refer to the phylogenetic tree below to answer questions 6 and 7.



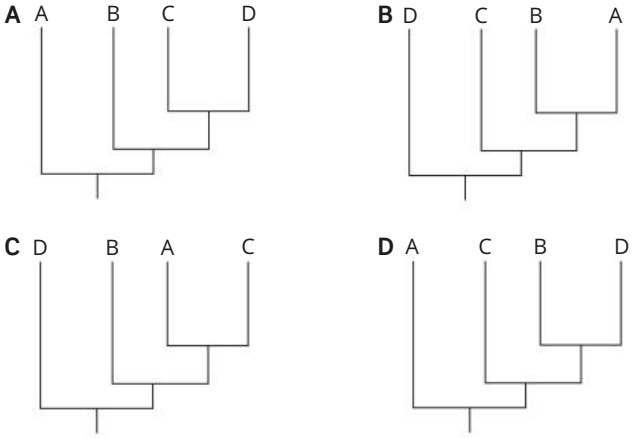
6 Select the position number that could be a common ancestor of both species P and T.

A 2
B 3
C 4
D 5

7 Select the two species that are most closely related to each other.

A P and Q
B Q and R
C R and S
D P and T

8 The family Felidae includes many species of felines. Three species in this family are *Catopuma badia* (species A), *Catopuma temminckii* (species B) and *Profelis aurata* (species C). Together these are known as golden cats. An outgroup of this clade is *Panthera leo* (species D). Assuming that these species are classified correctly, identify which of the phylogenetic trees most accurately shows the relationships between the species.



9 There are huge termite mounds throughout Central Australia. Termites are insects that consume large amounts of wood for food. They lack the enzymes needed to digest wood, and instead they have bacteria living in their intestines that break down the cellulose to glucose. The bacteria gain a stable environment in which to live while the termites gain access to the glucose from the cellulose. Identify the term that describes the relationship between the termites and the cellulose-digesting bacteria.

A parasitism
B mutualism
C competition
D commensalism

10 Identify the statement that best describes an organism's environment.

A It is the place where it lives.
B It is the ecosystem in which it lives.
C It is the set of physical factors that influence its survival.
D It is the sum of all of the living and non-living things that affect it.

Short answer

11 The New Mexico whiptail is a type of lizard found in the deserts of the American state of New Mexico. These lizards are the result of matings between western marbled whiptails (*Aspidoscelis tigris marmorata*) (pictured above right) and little striped whiptails (*Aspidoscelis inornata*). These matings are rare.

a State the term used to describe the offspring of a mating between two different species.
b Name a specific example of the offspring of a mating between two species that is unable to produce offspring.

12 **a** Define biodiversity.
b Explain why monitoring biodiversity is important.
c Outline why there is a need to conserve biodiversity.

13 In recent years the domestic dog, previously classified as *Canis familiaris,* and the Australian dingo, previously *Canis dingo*, were reclassified as *Canis lupus familiaris* and *Canis lupus dingo* respectively. This grouped these two types of canid into the same species as grey wolves, *Canis lupus lupus*.

a Explain the relationship that modern biologists have established between these three groups of organisms.
b Explain why early taxonomists classified domestic dogs, dingos and grey wolves as different species but modern taxonomists classify them as the same species.

14 A small flock of birds was caught in a cyclone and blown to an island previously uninhabited by that species. Conditions on the island were favourable for the birds.

a **i** Sketch a graph of the change in population over time until carrying capacity is reached if the birds use a *K*-selected reproductive strategy.
ii Explain how the graph would be different if the birds used an *r*-selected reproductive strategy.
b Birds lay eggs, which must be kept warm in order to hatch. Birds sit on their eggs to keep them warm. Bird hatchlings are unable to feed themselves. Explain whether birds are more likely to be *K*-strategists or *r*-strategists.

15 **a** Explain how taxonomy and cladistics differ.
b Distinguish between the Linnaean system of classification and modern cladistics.
c Define a clade.

16 The table shows the amino acid differences between seven species of animals for the protein cytochrome *c*.

Animo acid differences in cytochrome *c* protein

Species	A	B	C	D	E	F	G
A	0						
B	19	0					
C	27	31	0				
D	8	18	26	0			
E	33	36	41	31	0		
F	18	1	32	17	35	0	
G	13	13	29	14	28	12	0

Use the data to construct a phylogenetic tree of the seven species.

17 **a** Use Sprecht's modified classification system as shown in Table 2.1.2 on page 23 to classify the community shown in the image if the trees are about 25 m tall.

b State the further information that you would need in order to classify the ecosystem using Holdridge's system of classification.

c Identify two microhabitats that could be found in this ecosystem.

18 **a** Identify which of the following factors are abiotic:

- temperature
- predation
- rainfall
- wind speed
- competition
- soil type
- disease
- soil depth

b **i** Choose two of the abiotic factors and describe how they would change between the foot (base) and the apex (top) of a mountain.

ii Explain how these changes in abiotic factors would impact on the biodiversity and species abundance of the ecosystems on the top and at the foot of the mountain.

19 **a** Explain why knowledge of the types of species interactions that occur is necessary for understanding how to maintain biodiversity.

b Classify the following species interactions:

i senior male gorillas stop junior males from mating

ii a flea feeds on a dog

iii a cassowary eats fruits from various rainforest trees and then disperses the seeds in its droppings

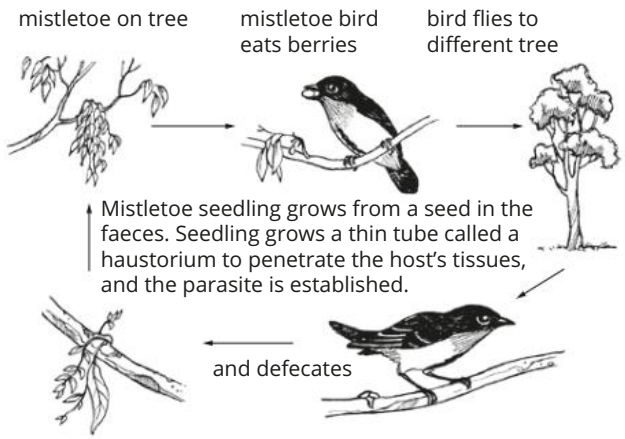
iv two magpies fight over a worm

v a lion steals the kill from a leopard

vi a hermit crab uses the discarded shell of a mollusc

c Nudibranchs such as the one pictured below are marine organisms. Some species of nudibranchs consume algae belonging to the group dinoflagellates, and maintain them within their tissues where they use sugars produced by the algae. Identify the possible relationships between the dinoflagellates and the nudibranchs. Describe the conditions needed for each relationship.

20 Mistletoe is a semi-parasitic plant that grows on eucalypts and some other native plants. The diagram below shows how a mistletoe bird is able to transfer mistletoe seeds to the surface of the branch of a new host tree.



a Name the relationship between the mistletoe bird and the mistletoe. Explain your answer.

b Explain whether the arrows in the diagram convey the same information as the arrows in a food chain.

c Name the relationship between the organisms in each of the following scenarios:

i Black walnut trees produce juglone from their roots, which inhibits the growth of other plants.

ii Oxpecker birds eat parasites from the skin of a zebra.

iii The gaudy leaf frog shelters from the sun under the leaves of the vermilliad plant.

iv A tapeworm lives in the digestive tract of a dog and obtains absorbs nutrients from the digested food.

21 A square metal frame 1 m × 1 m in size was thrown at random 20 times in each of two areas of alpine grassland. One area was fenced to exclude cattle and the other was unfenced. The abundance of four plant species was recorded in each 1 m × 1 m quadrat as the percentage area of the quadrat covered by the plant. (Since plants can overlap one another, these percentages do not necessarily add up to 100.) The values for the 20 replicate quadrats were averaged for each area. The results are shown in the table below.

Percentage cover of plant

Plant	Unfenced area	Fenced area
snow grass	45.6	67.4
silver snow daisy	2.4	41.7
scaly buttons	19.2	25.8
alpine star-bush	7.0	40.6

a Describe the overall difference between the two areas.

b Describe the differences between the abundance of the four species in each area.

c Propose reasons for the results.

22 Andrew wanted to test the effect of light intensity on the growth rate of grass plants. He placed five grass plants in full sunlight, five plants in the shade and five in darkness and measured their height each day.

a Identify the independent and dependent variables in this experiment.

b Explain why Andrew used five plants for each test.

c List two variables that would need to be controlled in this experiment.

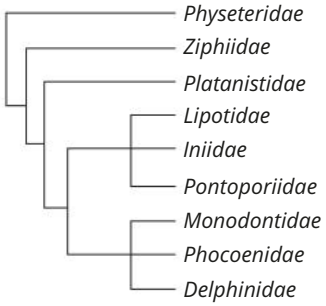
23 For many years, it was considered that giant pandas and red pandas were closely related. Both pandas live in cool, temperate areas near the Himalayas. Both have a false thumb, which is an extension of a wrist bone, and both have a diet that is primarily based on bamboo.

a Explain why it was once thought that the two types of pandas were close relations.

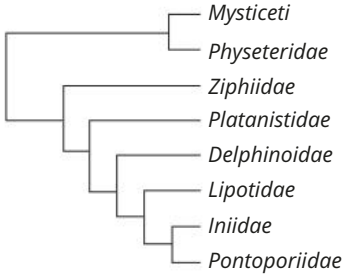
b DNA hybridisation studies have shown that in fact giant pandas are more closely related to bears, while red pandas are more closely related to racoons. Explain DNA hybridisation.

24 There are four families of river dolphin found in rivers around the world: *Pontoporiidae*, *Iniidae*, *Lipotidae* and *Platanistidae*. Two phylogenetic trees were constructed, using different types of evidence for an inferred relationship between river dolphins and toothed whales. One phylogenetic tree was constructed using structural morphology. The other phylogenetic tree was constructed using molecular sequences. Both are shown below.

Phylogenetic tree using morphology



Phylogenetic tree using molecular sequences



a State what material would be used to produce the phylogenetic tree constructed using molecular sequences.

b Using the phylogenetic tree constructed with molecular sequences, identify which other family of river dolphins is most closely related to *Platanistidae*.

25 A lake in Queensland has a variety of fish:

- 206 barramundi
- 304 golden perch
- 564 jungle perch
- 188 rainbow fish
- 947 spotted flagtail

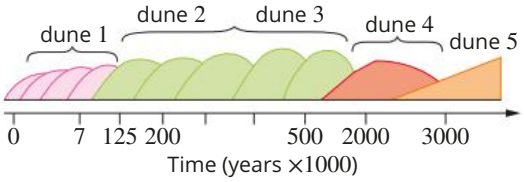
a Calculate the species diversity of the lake using Simpson's diversity index.

b Appraise your calculation.

The species diversity of a smaller, neighbouring lake in Queensland was also calculated. The Simpsons's diversity index of the second lake was calculated to be 0.5.

c Propose a reason for the difference in species diversity of the two lakes.

26 An area of land that covers a series of ancient sand dunes is proposed to be cleared for farmland. Either canola or wheat will be grown in the new fields. The area stretches across 5 km and covers several ancient dunes which range in age from 7000 years (dune 1) to 3 000 000 years old (dune 5).

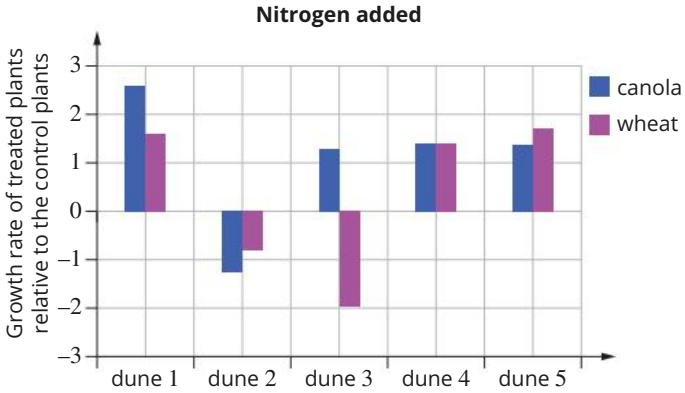


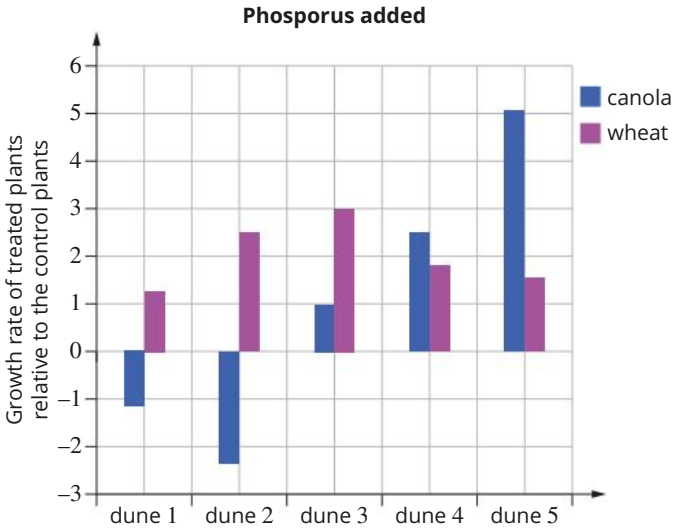
These dunes were formed during five periods of deposition. They have different soil qualities, as they were deposited under different conditions. Dunes 2 and 3 represent continuous deposition, but with differences in climate and weathering during and after deposition.

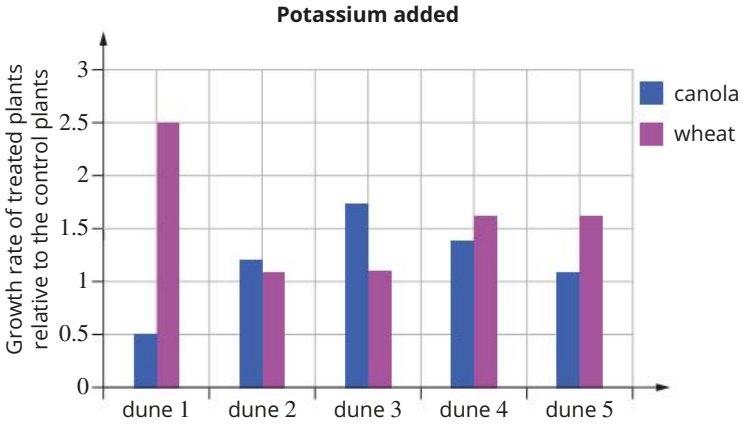
You are a scientist who has been consulted to make recommendations about which crops should be grown on each of the dune stages.

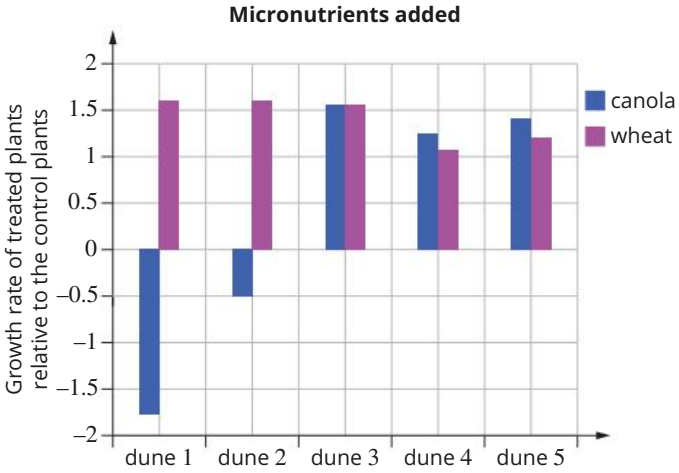
a Initially, you need to decide how to take your soil samples from the dunes for your future laboratory tests. Explain a suitable method for selecting the sites for the soil samples and state why more than one sample is needed.

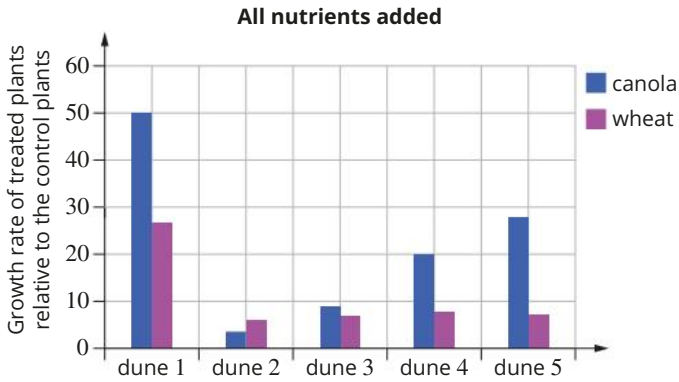
You perform a series of experiments where you grow both wheat and canola in soils taken from the five depositional areas. You treat the experimental groups with various nutrient solutions. For each nutrient solution (+ nitrogen, + phosphorus, + potassium, + micronutrients, + full nutrient) there is also a control set of plants that is completely untreated. A comparison between the growth rates of the nutrient-added plants and the controls was performed. The results of the series of experiments are shown in the following graphs.











b Identify the nutrient that has the most variable effect on the growth of canola plants.

c When comparing the data in each graph, it is difficult to ascertain which nutrients are having the largest effect. Explain why and state how this difficulty could be overcome.

d Examine the data carefully and, based on the data, make recommendations as to which crop should be grown. Justify your recommendations.

e Describe any further information that would be needed for a final recommendation about the crops to be planted and the treatments to be used.

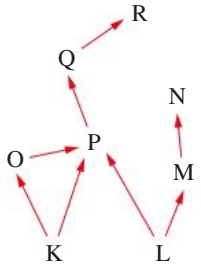
27 Female offspring formed from the rare matings of the western marbled whiptail (*Aspidoscelis tigris marmorata*) (pictured in question 11) and little striped whiptails (*Aspidoscelis inornata*) are viable and fertile but males are neither. These female offspring are commonly known as New Mexico whiptails. New Mexico whiptail females are able to reproduce by a form of asexual reproduction called parthenogenesis. All offspring are also female and are also able to reproduce by parthenogenesis. As a result of their ability to undergo asexual reproduction, New Mexico whiptail populations have been sustained over many decades. Some biologists, but not all, classify the New Mexico whiptails as a separate species (*Aspidoscelis neomexicana*). Discuss whether the New Mexico whiptail should be classified as a separate species.

28 Discuss the sampling methods used to collect data for your field research task. Evaluate the accuracy of the methods used and propose any changes that could be undertaken if further research were to be performed.

Topic 2 Ecosystem dynamics

Multiple choice

1 Using the food web shown, determine the correct description.



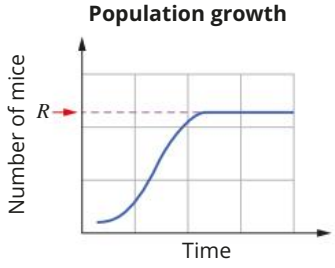
A K is a predator.
B M is a carnivore.
C Q is a herbivore.
D P is an omnivore.

2 The diagram below shows three populations that make up a food chain. If a new predator moves in that also feeds on fish, predict what might happen to the numbers in the three populations.

seagrass	→	fish	→	pelicans
population 1		population 2		population 3

A Numbers in all three populations decline.
B Numbers in population 2 decline, but those in 1 and 3 increase.
C Numbers in population 1 increase, but those in 2 and 3 decline.
D Numbers in population 2 decline, but those in 1 and 3 stay the same.

3 Identify the term that correctly describes the number of mice indicated by R.



A plateau number
B peak population
C carrying capacity
D population maximum

4 Without any limiting factors, growth of a population will be exponential. Limiting factors can be density-dependent or density-independent. Identify the example of a density-independent factor.

A crowding
B predation
C natural disasters
D competition for resources

5 Frogs can be used as an indicator of the health of a waterway. The pollution tolerances of a number of species of frog are shown in the table below.

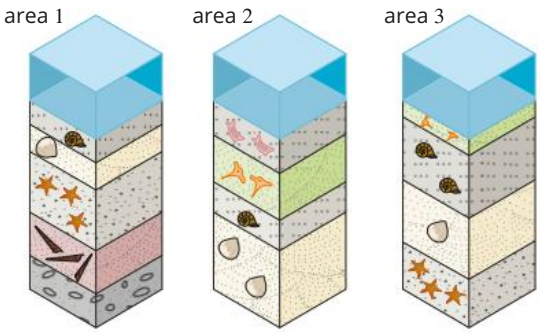
Pollution tolerance of selected frog species

Zero tolerance species	Low tolerance species	Medium tolerance species	High tolerance species
Pseudophryne australis	*Litoria phyllochroa*	*Litoria dentata*	*Limnodynastes peronii*
Pseudophryne bibroni	*Uperoliea laevigata*	*Litoria peronii*	*Crinia signifera*
	Limnodynastes dumerilii	*Litoria verreauxii*	

Identify the pair of species that could only occur in a waterway with no pollution.

A *Crinia signifera* and *Litoria dentata*
B *Limnodynastes dumerilii* and *Litoria peronii*
C *Pseudophryne australis* and *Uperoliea laevigata*
D *Limnodynastes dumerilii* and *Limnodynastes peronii*

6 Stratigraphic correlation indicates relative dates of rock layers by comparing their sequence from oldest (deepest) to youngest (closest to the surface). From the diagram below, identify which area has the oldest rocks and fossils.



A area 1
B area 2
C area 3
D They are all the same age.

7 Many lichens grow on the bark of trees. Identify the term used to describe the relationship between the lichens and the trees.

A symbiosis
B mutualism
C parasite–host
D commensalism

8 As part of an ecological study, the biomass of the organisms R, S, T, U, V and W in a community was estimated. The results are shown in the table below.

Biomass of six organisms within a community

Organism	R	S	T	U	V	W
Biomass (kg)	50	10	350	200	3000	75

Identify the most probable food chain.

A S → W → U → V
B T → V → R → S
C U → T → W → R
D V → T → W → S

9 The following food chain shows the relationship between four organisms:

grass → grasshopper → wolf spider → magpie

If the grass has 100 units of energy, calculate the amount of this energy that reaches the magpie.

A 1%
B 50%
C 10%
D 0.1%

10 A scientist is planning to estimate the size of an ibis population in Cairns parklands. Select the most appropriate sampling method.

A transect
B random quadrats
C percentage cover
D capture-tag-recapture

11 Select which of the following groups correctly shows the energy sources of photoautotrophs, chemoautotrophs and heterotrophs.

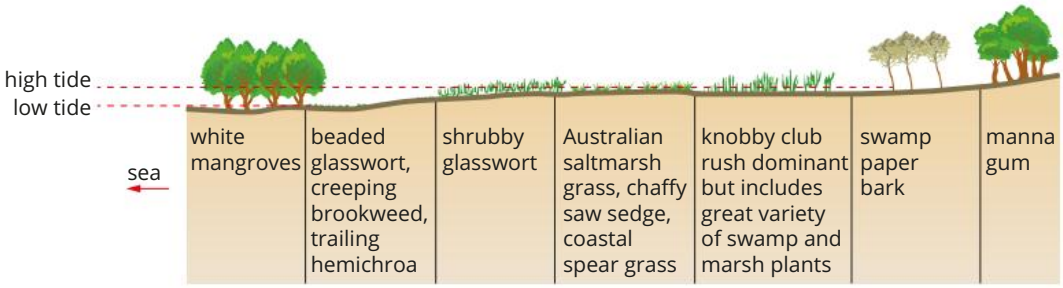
	Photoautotrophs	Chemoautotrophs	Heterotrophs
A	light	light	inorganic compounds
B	light	inorganic compounds	organic compounds
C	inorganic compounds	light	organic compounds
D	inorganic compounds	inorganic compounds	inorganic compounds

12 Identify which of the following are two of the most important abiotic factors affecting the distribution and species of Australian organisms.

A temperature and rainfall
B predators and food source
C humidity and light availability
D water availability and introduced species

Short answer

13 This diagram shows a transect of a saltmarsh. The plant species found in each zone marked in the transect are listed below the drawing.



a Explain the reason for the relatively sharp boundaries between the different species of plants found in the zones shown in the transect.

b The transect is an example of ecological succession. Explain ecological succession. Refer to the pioneer and climax communities.

14 Explain why light availability impacts the amount of biomass produced in an ecosystem.

15 Identify which organism is the producer by representing the following information as a biomass pyramid. Clearly identify the organism that is the producer and justify your choice.

Biomass of four organisms

Organism	Biomass
A	1000
B	10 000
C	10
D	1

16 **a** Distinguish between:

- **i** habitat and niche
- **ii** community and ecosystem
- **iii** obligate mutualism and facultative mutualism
- **iv** primary and secondary succession
- **v** density-dependent factors and density-independent factors
- **vi** fundamental niche and realised niche

b State the term used to describe the process that causes an organism to occupy its realised niche rather than its fundamental niche.

17 A study of the ecological niches of the giant and red pandas showed that the red panda preferred sparser forests than the giant panda. A study also showed that although the two species occupy the same habitat, the range of the red panda was much larger than that of the giant panda.

a Define ecological niche.

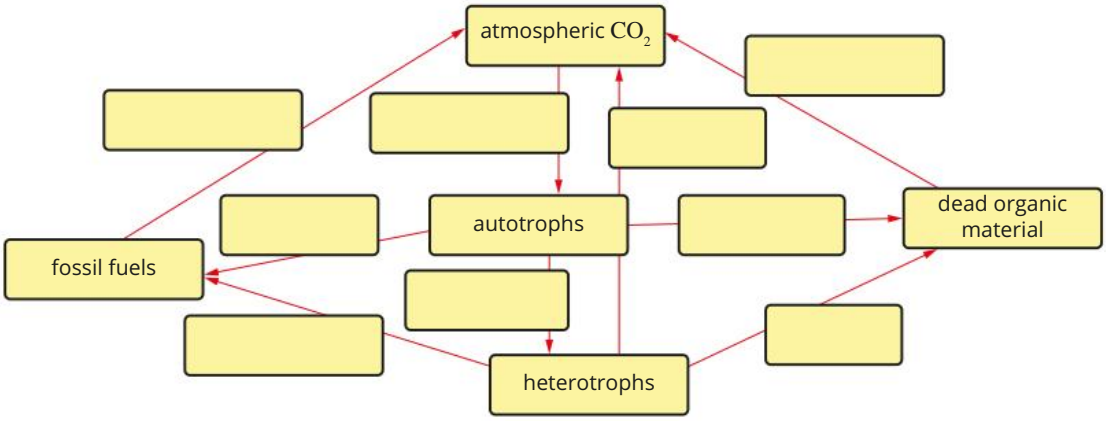
b Explain why the two species can live in the same area without competing.

---

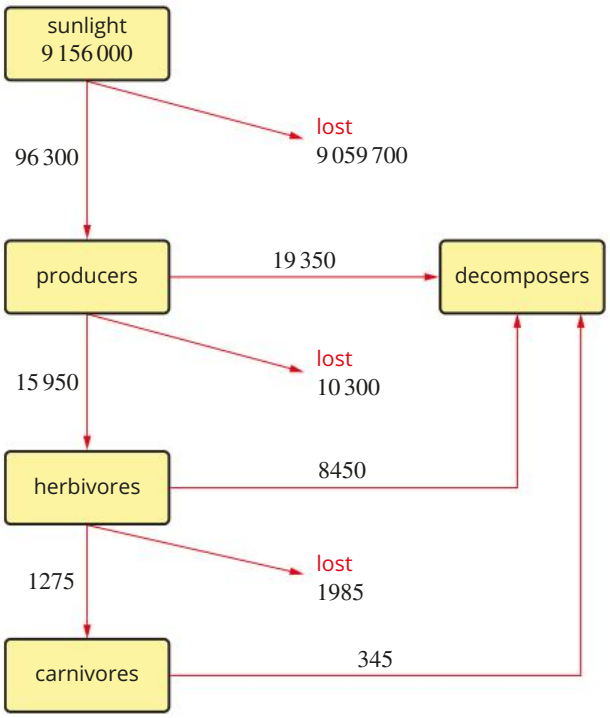
18 **a** Complete the flow chart of the carbon cycle by identifying the processes involved.

b Identify and explain the transformation that occurs to allow carbon to enter an ecosystem.

c Nitrogen is another important element that cycles through ecosystems. State the four types of bacteria that are involved in the nitrogen cycle and describe their roles.



19 The diagram shows the energy transformations in a small lake ecosystem over the period of a year in kJ m^{-2} y^{-1}.



a Calculate the percentage of light energy falling on the lake that is incorporated into the biomass of the producers.

b Predict what happens to the energy lost from the herbivores.

20 **a** Explain why habitat fragmentation leads to loss of biodiversity.

b Discuss the causes of habitat fragmentation.

21 **a** Create a table that compares the characteristics of pioneer species with those of the climax community.

b Use the table to explain why pioneer species are effective colonisers.

22 Examine the strata sequence below.

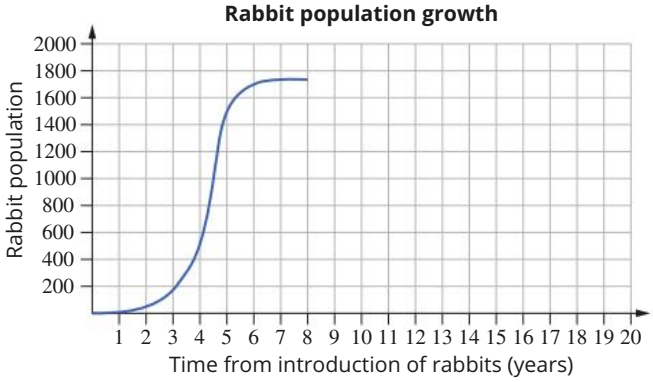
Details of strata

Stratum and thickness	Macrofossils	Microfossils	Sediment type
A 1.5 m	 	mainly grass pollens	conglomerate, cave infill
B 4 m	 	angiosperm and conifer pollens	river sediments
C 1.2 m	 	mostly freshwater diatoms and formaniferans, few grass and tree pollens	mudstone
D 0.4 m	fossil-free layer		volcanic ash
E 6 m	 	gingko and cycad pollens, fern spores	coal
F 10 m+	 	marine plankton including diatoms, formaniferans and coral larvae	marine sandstone

Discuss the changes that have taken place over time at this site. Justify your contentions with evidence from the fossil sequence.

23 Six rabbits were accidentally introduced onto a small island where they had not previously existed. The population grew over the next six years as shown in the graph below.

Rabbit population growth



a Describe the population growth over the first six years.

b Explain what occurred between the sixth and ninth years.

Over the next few years, the rabbit population was monitored. The results are shown in the table below.

Rabbit population growth, years 9 to 18

Year	Population
9	1725
10	1520
11	835
12	825
13	825
14	825
15	825
16	900
17	920
18	890

c i Plot the data onto a copy of the graph above.

ii Between years 9 and 18, rainfall to the island decreased significantly. Propose why the population decreased after year 9.

iii Identify two abiotic factors other than rainfall that could affect the island ecosystem.

d Hypothesise the effect of the introduction of the rabbits on the biodiversity of the island. Justify any proposals.

24 A flock of 70 cockatoos was observed over a period of three years. During that time the flock produced 60 live hatchlings but only 10 of them survived. Fifteen adults also died. Four birds joined the flock and eight left it.

a Calculate the size of the flock after the three years.

b Calculate the average rate of change in the cockatoo flock.

25 The range of species found in a waterway is a good indication of its health. As waterways become polluted, diversity declines as less tolerant species die out. A study was done to identify the fish species present in a small stream near a proposed site for a paper manufacturing plant. Chlorine bleach is used to whiten paper during the manufacturing process. Chlorine bleach has a pH of around 12, so there was concern about how this might affect the fish in the waterway. The normal pH of the stream was shown to be about 6.5. An experiment was performed to determine the pH-tolerance range of the fish living in the stream.

a Explain what is meant by the tolerance range of a species.

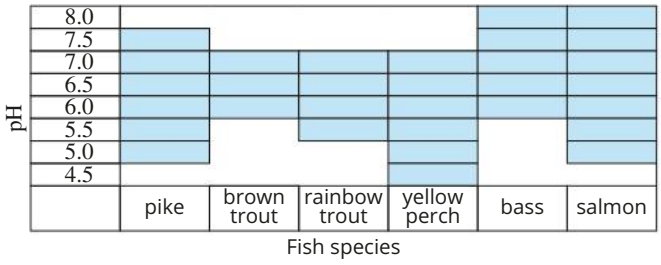
During the experiment, 500 members of each species were raised in ponds. Ten ponds were constructed. The first pond was maintained at a pH of 4.0, the second at 4.5, the third at 5.0 and so on. The last pond had a pH of 8.5. Fifty fish of each species were placed in each pond and their numbers monitored. After several weeks, the number of surviving fish was counted and conclusions about how they cope with various pHs was inferred.

b i Define inference.

ii Identify the independent variable in this experiment.

The results of the experiment are illustrated on the chart below.

pH tolerance ranges of several species of freshwater fish

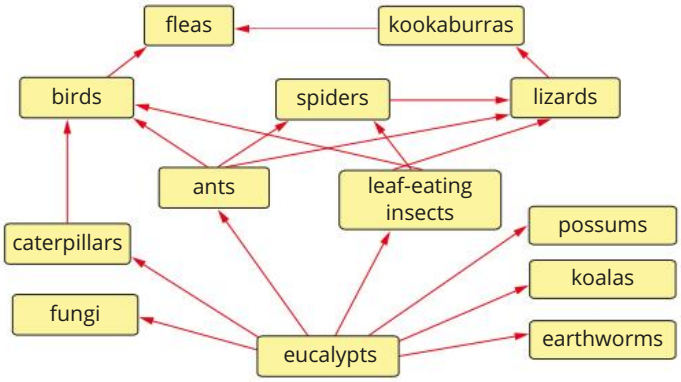


c Identify the species that is most sensitive to changes in pH.

Following this study, permission was granted for the factory to be built but the company was not permitted to pump wastes into the stream. All went well for three years until one night a tanker carrying bleach to the factory was involved in an accident and rolled into the stream where it leaked many litres of bleach raising the pH to 8.

d Predict the effect on the fish populations.

26 Study the following food web of a eucalypt forest.



a Identify the original source of energy for organisms in the food web.

b Name the producer(s) in this food web.

c For this food web, identify a:

i predator–prey relationship

ii parasite–host relationship

d Draw a pyramid of energy for this ecosystem.

e If the herbivores in this community have an energy content of 25 000 kJ, calculate the biomass of producers. Use the 10% rule for your calculations.

f Kookaburras are carnivores. Explain how they depend on producers.

g 'Food webs are more stable than food chains.' Explain why this is so, using an example from the food web shown above.

27 The greater glider (*Petauroides volans*) is a small gliding marsupial found along the east coast of Australia, from southern Queensland to Victoria. They eat eucalypt leaves and are known to feed on eucalypt buds and flowers. The greater glider prefers habitats that contain mature gum trees (including *Eucalyptus viminalis, Eucalyptus dalrympleana* and *Eucalyptus obliqua*), nesting in multiple tree hollows. The species is currently classified as vulnerable.

a Define the greater glider's ecological niche in terms of its feeding.

Recent urban growth in and around Brisbane has seen urban areas of Brisbane join to the Sunshine Coast, Logan, the Gold Coast and Ipswich.

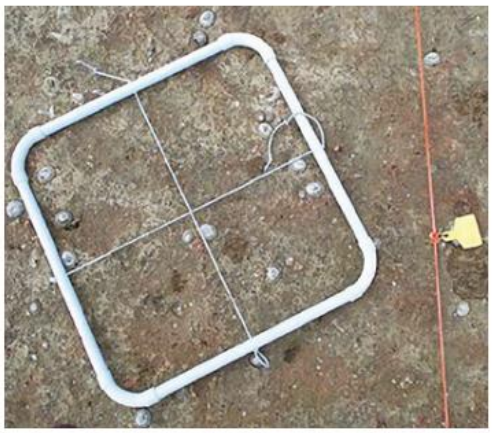
b Predict what impact urban development will have on the population of greater gliders in the Greater Brisbane area.

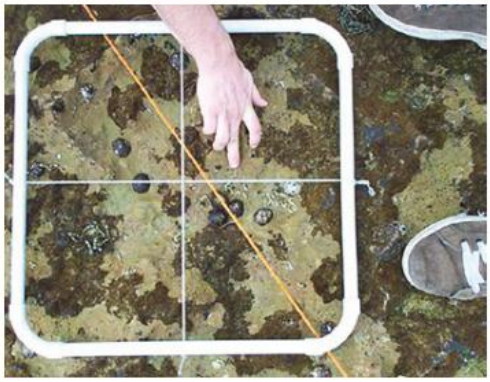
c Explain your prediction in relation to:

i speed of ecosystem change

ii duration of ecosystem change.

28 Students wanted to investigate the distribution patterns of periwinkles and brown algae at various distances from the high-tide mark along an intertidal rocky shore. First, they made a transect line, starting from the high tide mark (a). The students placed a quadrat (0.5 m × 0.5 m) at the 0 m mark on their transect. They counted the number of periwinkles in the quadrat and also determined the percentage of the quadrat covered by brown algae (b). The results of the survey are shown in the following table.





Distribution of periwinkle and percentage cover of brown algae at different distances from the high-tide mark

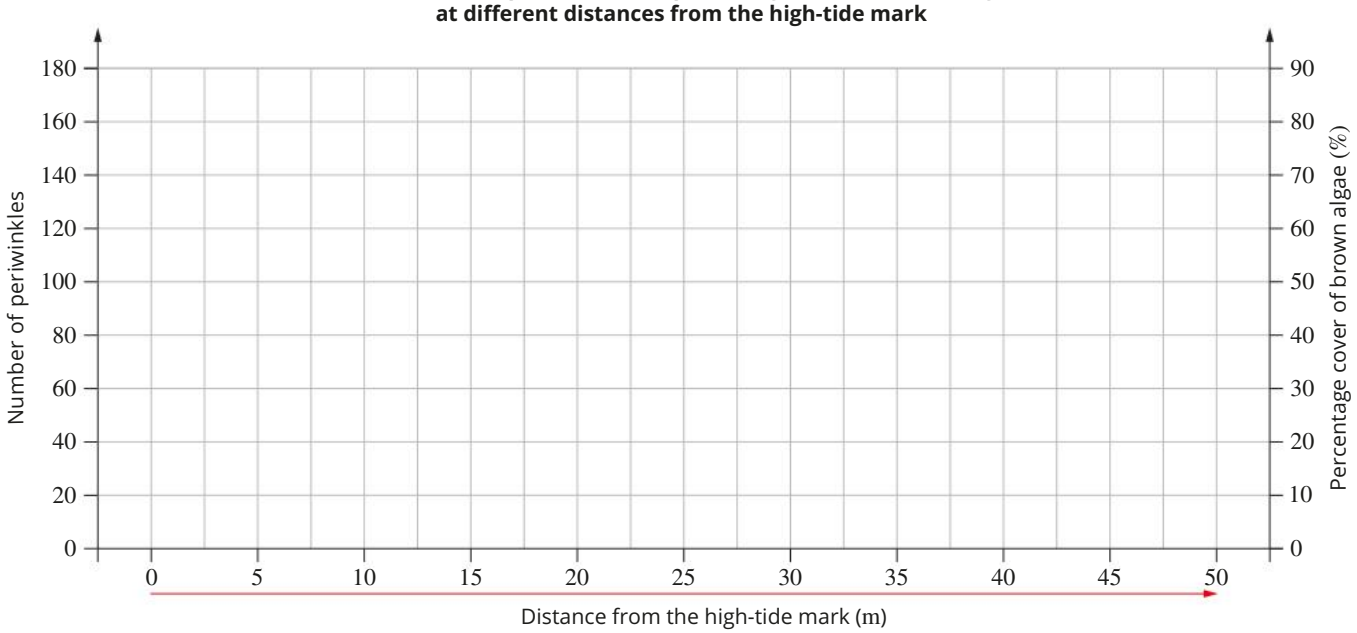
Distance from high-tide mark (m)	Number of periwinkles in the quadrat	Percentage cover of brown algae in quadrat (%)
0	147	0
5	162	4
10	20	13
15	0	7
20	1	9
25	10	50
30	1	8
35	0	0
40	0	0
45	0	0
50	0	0

a Plot the results of the survey on a copy of the graph below.

b The students did some research on brown algae. They discovered that some brown algae contain phlorotannins, which act as a chemical defence against grazing by organisms such as periwinkles. The students hypothesised that the change in the density of periwinkles along the transect from the high-tide mark to the 50 m mark is due to the inability of periwinkles to graze on brown algae. Evaluate the students' hypothesis.

c Propose how the reliability of the results could be improved.

Distribution of periwinkle and percentage cover of brown algae at different distances from the high-tide mark



29 Jenny captured seven koalas in the Port Stephens area, tagged them and released them back into the wild. Some months later, Jenny recaptured 10 koalas and found that two of them were tagged.

a Identify the name of the technique Jenny used.

b Calculate the total abundance of koalas in the area. Use the Lincoln index and show all working.

c Justify why Jenny chose this method for calculating the abundance of koalas.

d Assume the population consists of all breeding pairs and any unmated koalas emigrate from the area. If each pair produces one offspring, which is ready to breed at one year old, and the death rate of the population is 10%, calculate the population after three years. Show all working. (Round mortality to the nearest whole number.)

30 Throughout Europe and America bee colonies have shown a dramatic rise in the incidence of colony collapse disorder (CCD). When this occurs, most of the worker bees disappear almost overnight, leaving the queen, the larvae and a few nursemaid workers behind. In some areas, hive losses are as great as 50%. Not only has this caused economic loss to the beekeepers, many farms that rely on the bees to pollinate their crops have also suffered severe losses. Bees are the predominant method of pollination, and therefore fundamental to the reproduction of crops and to the local ecosystems.

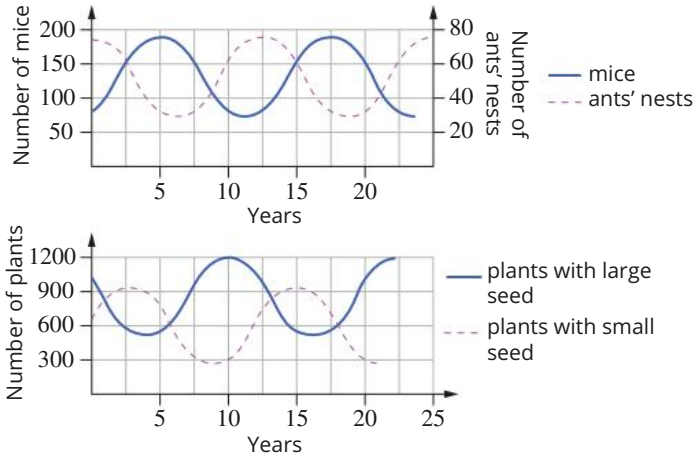
a Identify the type of species these bees are classified as.

Three causes have been suggested for CCD: the use of pesticides and fungicides on the crops from which the bees collect pollen, malnutrition due to the lack of wild flowers in areas where most of the land is covered in crops, and disease, especially the parasitic mite, *Varroa*, which has become common in both Europe and America. This parasite is not found in Australia. Bee colonies in Australia have not shown signs of CCD.

b Draw a conclusion about the causes of CCD.

31 Scientists noticed that there seemed to be a relationship between the population of a native mouse and the number of ant nests in a particular ecosystem. They could find no direct connection between the two organisms. For example, the mice did not eat the ants or compete with them for food or nesting space. They then noticed that there seemed to be a relationship between the different foods favoured by the mice and the ants. The mice ate large seeds from a particular plant and the ants ate small seeds from a different plant. The graphs below show the changes in population of the mice and the number of ants' nests, and plants with large seeds and those with small seeds.

Relationships between number of mice, ants' nests and plants



a Propose a possible relationship between the two species of plants.

b Propose a research question to explain the fluctuations in the population of mice and the number of ants' nests in the ecosystem. Explain, using the evidence from the graphs, why you believe this is a reasonable research question.

UNIT 4 Heredity and continuity of life

TOPIC 1 DNA, genes and the continuity of life

TOPIC 2 Continuity of life on Earth

Unit 4 objectives

Students will:

1. describe and explain DNA, genes and the continuity of life, and the continuity of life on Earth
2. apply understanding of DNA, genes and the continuity of life, and the continuity of life on Earth
3. analyse evidence about DNA, genes and the continuity of life, and the continuity of life on Earth
4. interpret evidence about DNA, genes and the continuity of life, and the continuity of life on Earth
5. investigate phenomena associated with DNA, genes and the continuity of life, and the continuity of life on Earth
6. evaluate processes, claims and conclusions about DNA, genes and the continuity of life, and the continuity of life on Earth
7. communicate understandings, findings, arguments and conclusions about DNA, genes and the continuity of life, and the continuity of life on Earth.

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CHAPTER 05

DNA and replication

The molecular basis for inheritance is the DNA molecule. Its structure was determined by scientists in the 1950s, and it became one of the most significant discoveries of the 20th century. Nucleic acids have the unique ability to direct their own replication and pass on the chemical code for particular characteristics to offspring. This chemical language dictates the synthesis of proteins to determine the biochemical, physiological and behavioural traits of an organism. When the integrity of the replication process is altered through mutation, new forms of genes are introduced to the gene pool. An understanding of the structure and function of DNA and its regulation of protein synthesis has led to the development of new technologies and the ability to potentially control which genes are passed onto the next generations.

By the end of this chapter, you will be able to describe the structure of the nucleic acids DNA and RNA, and their synthesis through condensation polymerisation. You will also understand the role of these nucleic acids as information molecules that encode instructions for protein synthesis, and the steps in eukaryotic and prokaryotic gene expression: transcription, RNA processing and translation. You will have explored gene structure and analysed the distinction between structural and regulatory genes and have an understanding of the regulation of gene transcription by transcriptional factors. You will be able to identify different types of mutations and analyse a genetic code to predict the effect of genetic changes due to transcription errors.

Syllabus subject matter

WS 4.1.1

Topic 1 • DNA, genes and the continuity of life

DNA STRUCTURE AND REPLICATION

- understand that deoxyribonucleic acid (DNA) is a double-stranded molecule that occurs bound to proteins (histones) in chromosomes in the nucleus, and as unbound circular DNA in the cytosol of prokaryotes, and in the mitochondria and chloroplasts of eukaryotic cells
- recall the structure of DNA, including:
  - nucleotide composition
  - complementary base pairing
  - weak, base-specific hydrogen bonds between DNA strands.

GENE EXPRESSION

- define the terms genome and gene
- understand that genes include ‘coding’ (exons) and ‘noncoding’ DNA (which includes a variety of transcribed proteins: functional RNA (i.e. tRNA), centromeres, telomeres and introns. Recognise that many functions of ‘noncoding’ DNA are yet to be determined)

- explain the process of protein synthesis in terms of:
  - transcription of a gene into messenger RNA in the nucleus
  - translation of mRNA into an amino acid sequence at the ribosome (refer to transfer RNA, codons and anticodons)
- recognise that the purpose of gene expression is to synthesise a functional gene product (protein or functional RNA); that the process can be regulated and is used by all known life
- identify that there are factors that regulate the phenotypic expression of genes
  - during transcription and translation (proteins that bind to specific DNA sequences)
  - through the products of other genes
  - via environmental exposure (consider the twin methodology in epigenetic studies)
- recognise that differential gene expression, controlled by transcription factors, regulates cell differentiation for tissue formation and morphology
- recall an example of a transcription factor gene that regulates morphology (*Hox* transcription factor family) and cell differentiation (sex-determining region Y).

MUTATIONS

- identify how mutations in genes and chromosomes* can result from errors in:
  - DNA replication (point and frameshift mutation)
  - cell division (non-disjunction)
  - damage by mutagens (physical including UV radiation, ionising radiation and heat and chemical).

* The greyed out sections of this dot point are addressed specifically in Chapter 7.

SCIENCE AS A HUMAN ENDEAVOUR

Understand the development of the double-helix model through the contributions of James Watson, Francis Crick and Rosalind Franklin.

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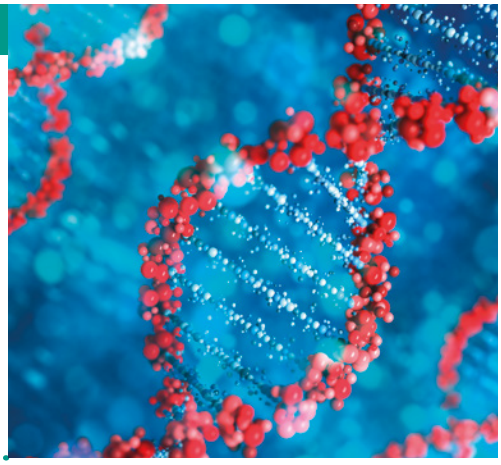
5.1 DNA structure and genetic code

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- describe the structure of the nucleic acids (DNA and RNA)
- understand that DNA is a double-stranded molecule that occurs bound to proteins in chromosomes in the nucleus, and as unbound circular DNA in prokaryotes and the mitochondria and chloroplasts of eukaryotic cells
- recall the structure of DNA including nucleotide composition and the complementary base pairing
- understand the difference in nucleotide composition and structure between RNA and DNA
- understand the role and structure of chromosomes
- recall the types of chromosomes.



DNA is a nucleic acid macromolecule that carries hereditary information from one generation to the next and directs the cell's activities. The genetic code for inherited traits is carried on the **chromosomes** in cells in sections of DNA called **genes**. In this module, you will learn about the structure and function of DNA and RNA.

NUCLEIC ACIDS—DNA AND RNA

Nucleic acids are organic biomolecules that store and transmit inherited characteristics of organisms. Specifically, nucleic acids encode instructions for the synthesis of **proteins**. The expression of the genetic code to produce proteins will be explored further in Module 5.2.

There are two types of nucleic acids:

- **Deoxyribonucleic acid (DNA)** carries the inheritable genetic code for the control of cell activities and **protein synthesis**. It has a double-stranded helix (spiral) structure (Figure 5.1.1) and carries the instructions that code for the production of RNA. DNA is able to self-replicate.

  In prokaryotes, DNA is found in the cytoplasm. In the cells of all eukaryotes, most of the DNA is found in the nucleus. There is also mitochondrial DNA (mtDNA) in the mitochondria of all eukaryotic cells and chloroplast DNA (cpDNA) in the chloroplasts of photosynthetic eukaryotes. The DNA in the mitochondria and chloroplasts of eukaryotic cells is in the form of unbound, circular DNA.
- **Ribonucleic acid (RNA)** is single-stranded. The various forms of RNA use the genetic code to produce proteins, which are the products of gene expression. Each type of RNA performs a different function. One type (messenger RNA) carries a copy of a DNA sequence, while another type (transfer RNA) has the ability to 'read' and translate the DNA information. RNA plays a major role in the process of protein synthesis.

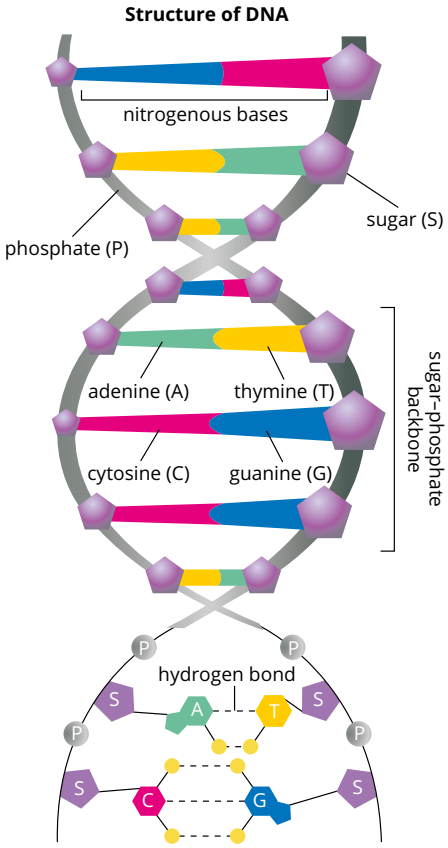


FIGURE 5.1.1 The structure of the DNA double-helix biomolecule. The helical structure of DNA is formed by two strands of complementary nitrogenous bases that are joined by hydrogen bonds. Each side of the helix is comprised of deoxyribose sugar and phosphate molecules, known as the sugar–phosphate backbone.

Nucleotides

Nucleic acids are polymers, made up of repeated subunit **monomers** or **nucleotides**. Nucleotides are the chemical building blocks of DNA and RNA.

A single nucleotide consists of three units:

- a phosphate group—the same in all nucleotides
- a five-carbon (pentose) sugar:
  - **deoxyribose** in DNA nucleotides
  - **ribose** in RNA nucleotides
- a nitrogenous (nitrogen-containing) **base**.

The five carbon atoms in a pentose sugar molecule are labelled 1' to 5'. In a single nucleotide, the phosphate is always attached to the 5' carbon and the base is always attached to the 1' carbon (Figure 5.1.2).

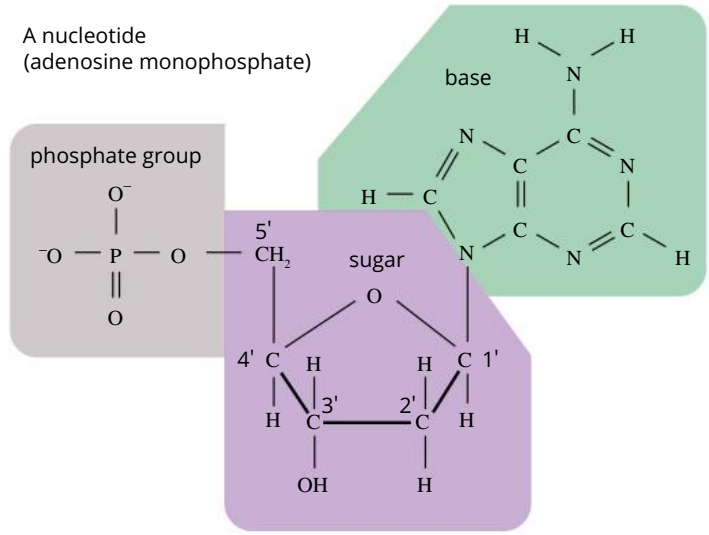


FIGURE 5.1.2 The structure of a DNA nucleotide, showing the phosphate group, the five-carbon sugar and the nitrogenous base adenine (A).

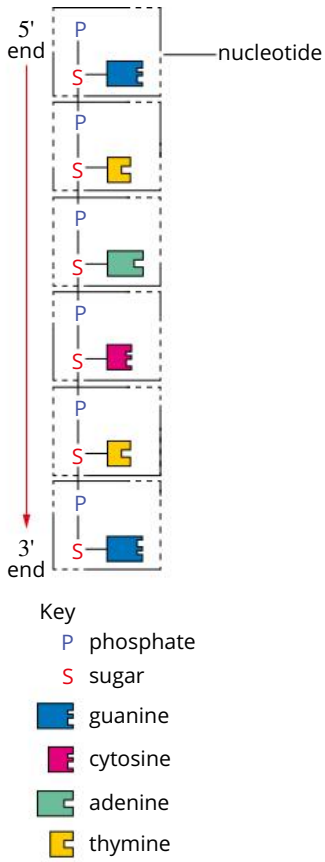


FIGURE 5.1.3 A single polynucleotide chain. Individual nucleotides (shown in boxes) are joined by phosphodiester bonds.

Polynucleotide chains

Nucleotides join together to form a polynucleotide chain, or strand (Figure 5.1.3). When nucleotides join, the ribose sugar and the phosphate group form a long-chain polymer that is the backbone of the DNA molecule. This process is called **condensation polymerisation**. During condensation polymerisation, a water molecule is released each time the phosphate and sugar monomers combine and a covalent bond (phosphodiester bond) forms, joining the nucleotides together.

The ends of the resulting polynucleotide chain are referred to as the 5' and 3' ends (pronounced 'five prime' and 'three prime'). At the 5' end of the polynucleotide strand, the fifth carbon atom is free. At the 3' end, the third carbon atom is free. DNA and RNA are synthesised in the 5' to 3' direction.

Nitrogenous bases

Attached to the ribose sugar within every nucleotide is a nitrogenous base. There are five different **nitrogenous bases**:

- **adenine (A)**
- **guanine (G)**
- **cytosine (C)**
- **thymine (T)**—in DNA only
- **uracil (U)**—in RNA only.

These five nitrogenous bases can be categorised into one of two groups based on their structure (Figure 5.1.4). **Pyrimidines** (C, T and U) have one ring in their structure. **Purines** (A and G) have two rings in their structure.

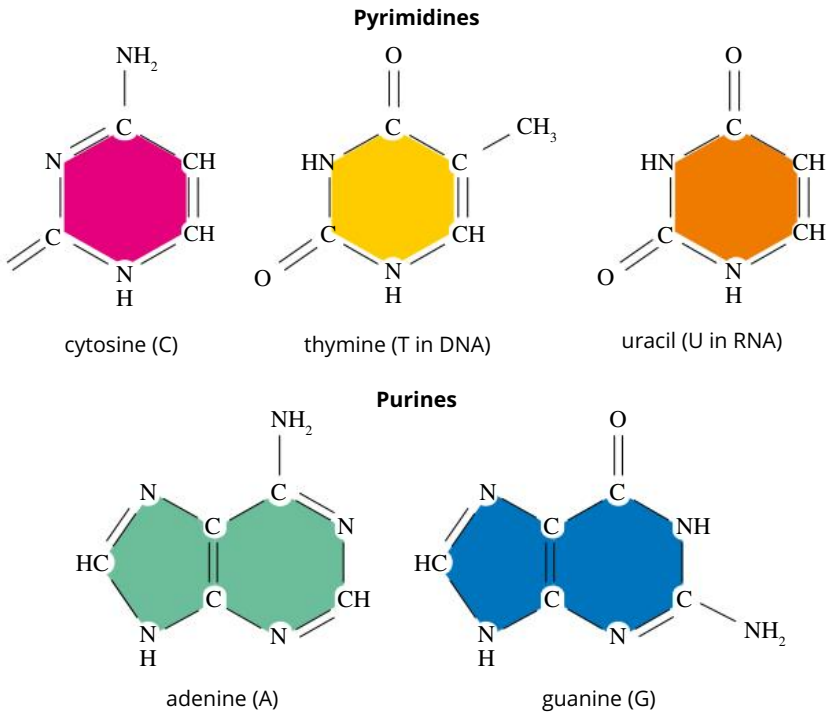


FIGURE 5.1.4 The structure of pyrimidines (cytosine, thymine and uracil) and purines (adenine and guanine).

THE STRUCTURE OF DNA

DNA is double-stranded. It consists of two chains or strands of nucleotides twisted into a **double-helix** structure. The primary structure of DNA is a single strand of polynucleotides, which consists of a specific sequence of nitrogenous bases (A, T, C and G). The opposing nitrogenous bases from two strands of DNA are joined by hydrogen bonds. These hydrogen bonds stabilise the secondary structure of the DNA and form the double helix (Figure 5.1.5).

When DNA is uncoiled, the two strands can be represented as a ladder. The sides of the ladder consist of the sugar–phosphate backbone. The two strands of a DNA molecule are **antiparallel**, meaning that they run in opposite directions. The 3' end of one strand matches up with the 5' end of the other strand. The rungs of the DNA ladder are the nitrogenous bases of each nucleotide (Figure 5.1.6). The nitrogenous bases can occur in any order within the strand.

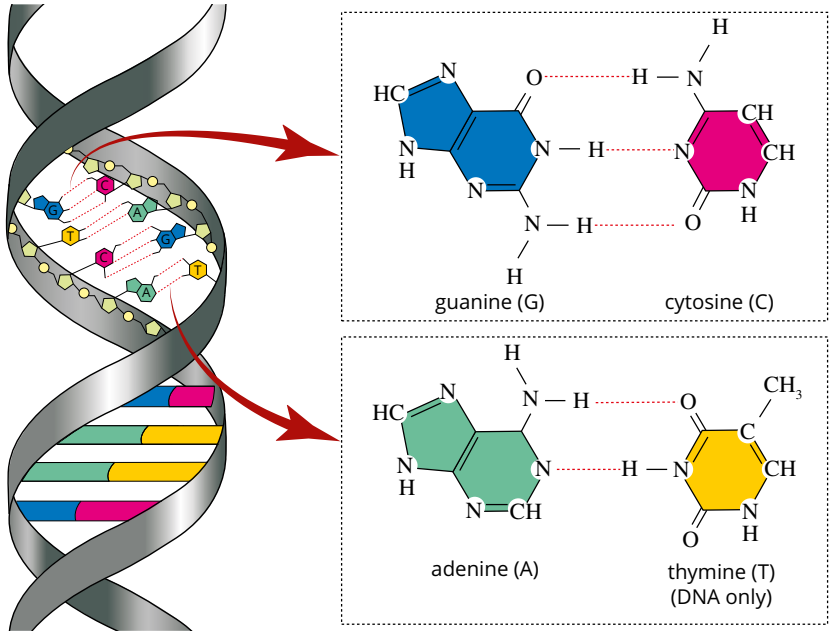


FIGURE 5.1.5 The helical structure of DNA. Two complementary strands form a double helix joined by base pairs guanine (G) and cytosine (C), and adenine (A) and thymine (T).

Chemical studies show that the concentration of adenine in a DNA molecule is always equal to that of thymine, and that the concentration of guanine is always equal to that of cytosine. These observations are explained by a direct pairing between A and T and between G and C in the DNA molecule. This pairing of nitrogenous bases in DNA molecules is known as **complementary base pairing**.

In complementary base pairing:

- the purine adenine (A) always pairs with the pyrimidine thymine (T), held together with two weak hydrogen bonds
- the purine guanine (G) always pairs with the pyrimidine cytosine (C), held together with three weak hydrogen bonds.

The diameter of the DNA double helix is approximately 2.0 nanometres and there are 10–10.5 pairs of nucleotide bases in each twist of the helix.

WS 4.1.2

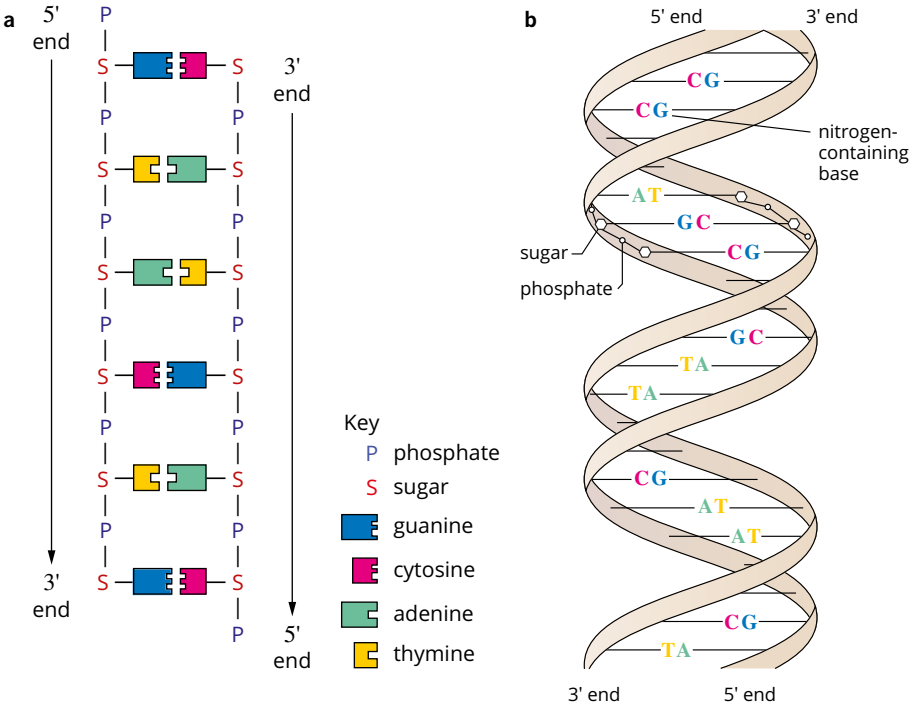


FIGURE 5.1.6 Two-dimensional representations of the DNA molecule. (a) Hydrogen bonds between the complementary base pairs hold the two antiparallel polynucleotide strands together. (b) The structure of DNA, showing the two complementary strands forming the double helix.

Eukaryotic versus prokaryotic DNA

In eukaryotic cells, DNA is found in the nucleus, where it is tightly coiled up to form structures called chromosomes. In prokaryotic cells, DNA is found in the nucleoid region as a large circular chromosome and small circular molecules called **plasmids** (Figure 5.1.7).

Eukaryotic cells are more complex than prokaryotic cells (Figures 5.1.8, 5.1.9 and 5.1.10). Eukaryotic cells have an average of 25 times more DNA than prokaryotic cells. Because of this, the processes involved in DNA replication are much slower in eukaryotic cells. Some bacterial cells take just 40 minutes to replicate their DNA, while in some animals this can take up to 10 hours.

FIGURE 5.1.7 Coloured transmission electron micrograph of plasmids from *E. coli* bacteria. Plasmids are small, circular DNA molecules that are commonly found in bacteria.

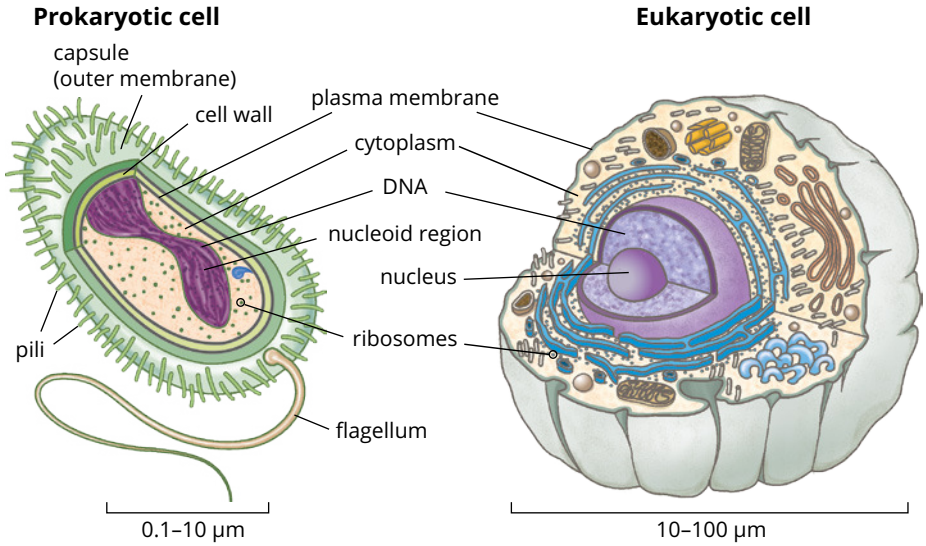


FIGURE 5.1.8 Prokaryotic cell compared to a eukaryotic cell. Prokaryotic cells and the processes involved in their DNA replication and gene expression are generally much simpler than in eukaryotic cells.

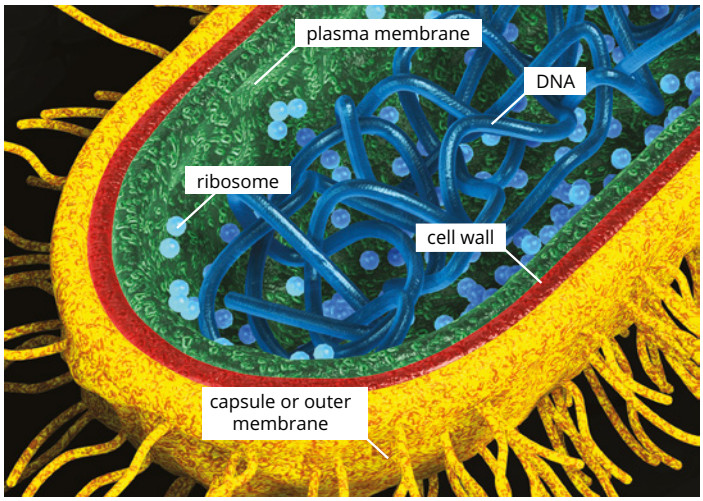


FIGURE 5.1.9 The inner structures of a prokaryotic cell. The pili and capsule are shown in yellow, the cell wall in red, the plasma membrane in green, the ribosomes in light blue and the DNA in dark blue.

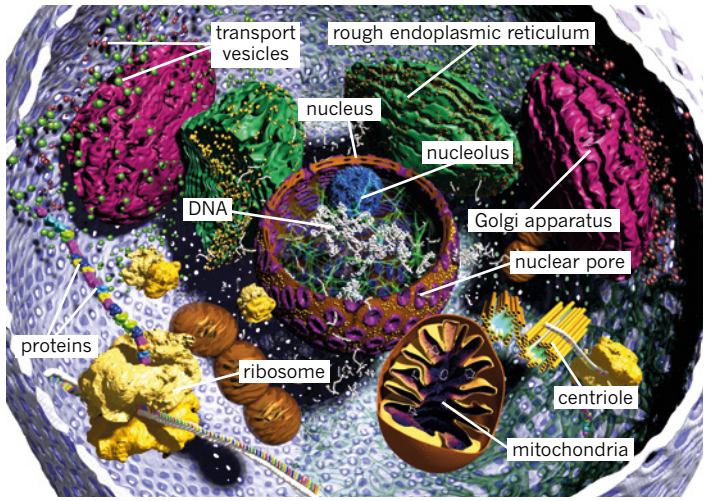


FIGURE 5.1.10 The inner structures of a eukaryotic cell include the nucleus (centre) which has a membrane with nuclear pores (purple). Found inside the nucleus is the DNA (genetic material, white) and the nucleolus (blue).

PA 4.1.1

THE STRUCTURE OF RNA

Unlike DNA, RNA is usually found as a single strand, sometimes folded onto itself. RNA molecules are usually much shorter than DNA molecules. The nucleotides of RNA have the same basic structure as those of DNA, with a few differences. DNA contains deoxyribose sugar, while RNA contains ribose sugar. The nitrogenous base thymine in DNA is replaced by uracil in RNA. Both of these bases pair with adenine (Figure 5.1.11). Uracil is more stable in single-stranded polynucleotides. Table 5.1.1 (page 254) summarises and highlights the differences between the structure of DNA and RNA.

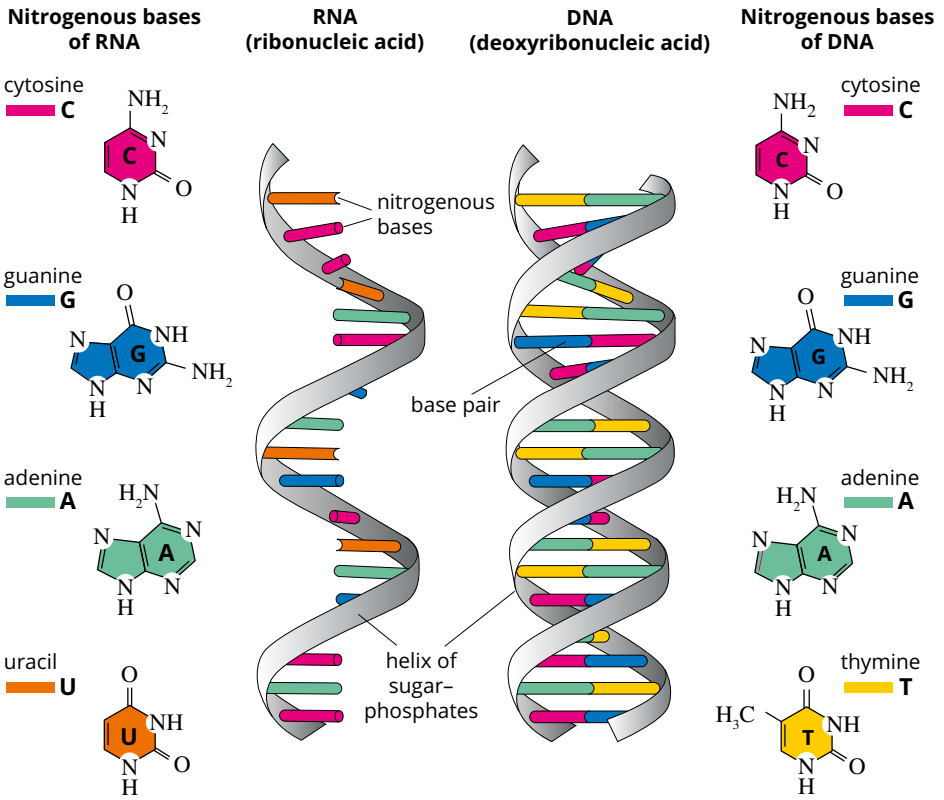


FIGURE 5.1.11 Comparison of the structures of RNA and DNA. RNA is single-stranded and DNA is double-stranded. Both molecules are made up of nitrogenous bases and a sugar–phosphate backbone, but in RNA ribose sugar replaces deoxyribose sugar and uracil replaces thymine.

In eukaryotic cells, RNA molecules are formed in the nucleus but pass into the cytoplasm for protein synthesis. There are three main forms of RNA. Each form of RNA has a specific role in protein synthesis.

- **Messenger RNA (mRNA)** carries a copy of the DNA's nucleotide sequence to be translated into proteins (Figure 5.1.12). The processes of transcription and translation will be discussed in Module 5.2.
- **Ribosomal RNA (rRNA)** forms ribosomes, the site of translation of the mRNA into proteins (Figure 5.1.13). The synthesis of proteins will be explained in Module 5.2.
- **Transfer RNA (tRNA)** carries amino acids to the appropriate positions on the mRNA by matching its anticodon sequence to the complementary sequence in the mRNA. The amino acids transferred by tRNA build the polypeptide chain during translation (Figure 5.1.14). This will be explored in detail in Module 5.2.

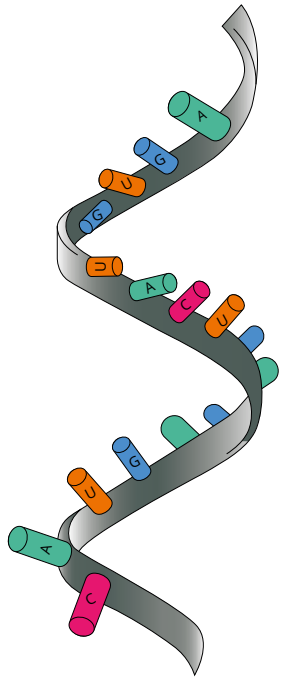


FIGURE 5.1.12 Messenger RNA.

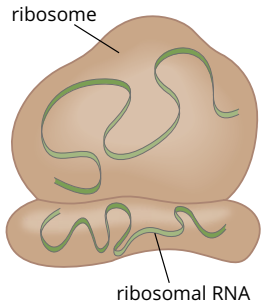


FIGURE 5.1.13 Ribosomal RNA.

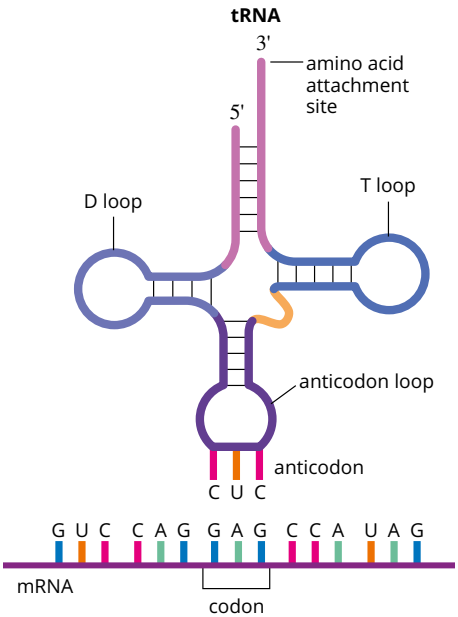
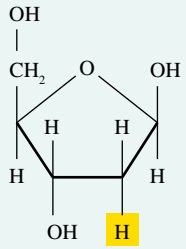
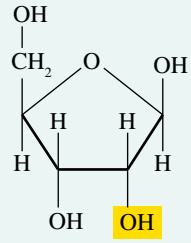
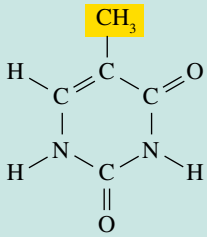
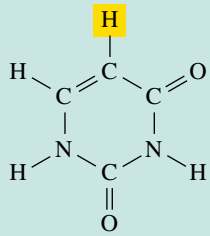
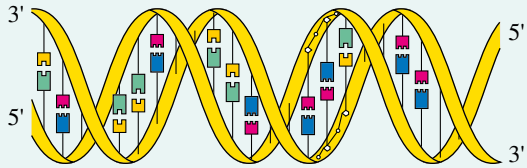
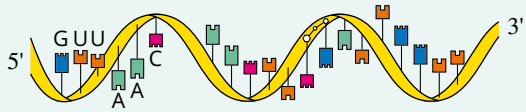


FIGURE 5.1.14 Transfer RNA.

TABLE 5.1.1 A summary of the differences between DNA and RNA

	DNA	RNA
relative length	long	short
sugar	deoxyribose  deoxyribose in DNA	ribose  ribose in RNA
bases	adenine cytosine guanine thymine  thymine in DNA	adenine cytosine guanine uracil  uracil in RNA
strands	double  	usually single 
base pairing	adenine–thymine cytosine–guanine	adenine–uracil cytosine–guanine

CHROMOSOMES

Structure of chromosomes

In eukaryotes, the DNA is coiled around small proteins called histones, forming a particle about 10 nm in diameter, called a nucleosome. Figure 5.1.15 shows DNA and associated proteins located in chromosomes. The eukaryotic chromosome shown in the image is at a stage in cell division where it is slightly condensed. The diagram shows a section of the chromosome unravelled to show how DNA is wrapped within it. The DNA is coiled and supercoiled around structural proteins.

Nucleosomes give the DNA strand the appearance of a string of beads. This arrangement packages the DNA efficiently and protects it from enzymatic degradation.

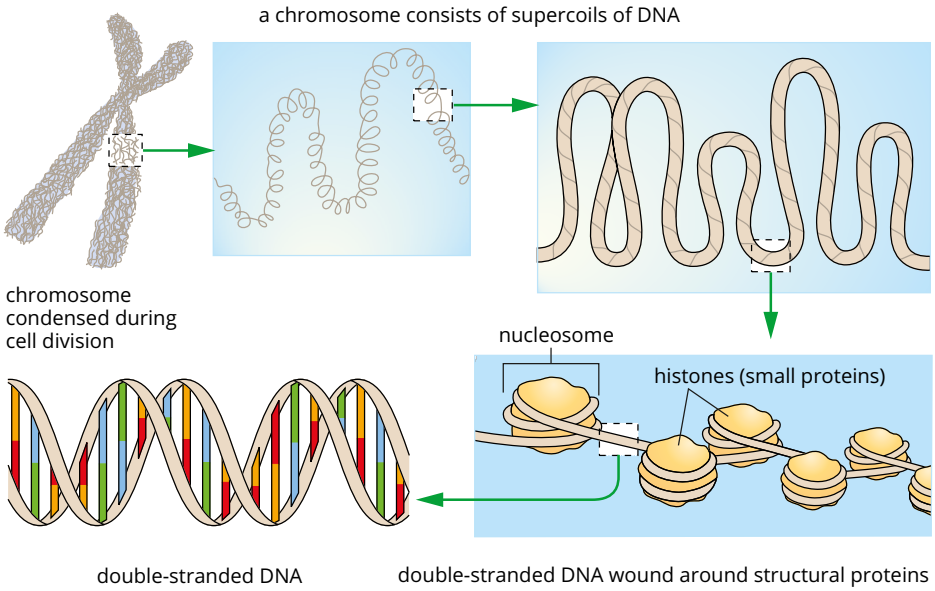


FIGURE 5.1.15 A chromosome unwound to reveal the complex substructure of supercoils of DNA. Each coil consists of histones that are structural proteins, which package and order the DNA into structural units called nucleosomes. Further unwinding of a nucleosome reveals the double-stranded DNA structure.

Types of chromosomes

Each eukaryotic chromosome has a constriction point called the centromere, which divides the chromosomes into two sections. The regions on either side of the centromere are referred to as the chromosome arms (Figure 5.1.16). The shorter arm is called the 'p arm' and the longer arm is called the 'q arm'. Photographs or diagrams of chromosomes are always arranged so that the p arm is at the top.

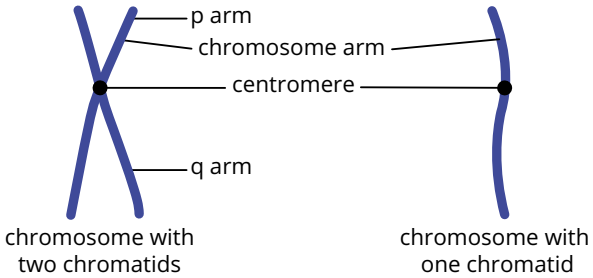


FIGURE 5.1.16 Parts of a chromosome.

> **i** The names of the types of centromeres are derived from Greek:
> - meta = in the middle
> - submeta = below the middle
> - acro = topmost, at the extremity
> - telo = end, terminus.

There are four major types of chromosomes, based on the position of the centromere on the chromosomes as they appear during metaphase (Figure 5.1.17):

- Metacentric chromosomes have the centromere centrally positioned, giving arms of equal length.
- Submetacentric chromosomes have the centromere towards one end, resulting in arms of unequal length, with a long arm approximately twice the length of the short arm.
- Acrocentric chromosomes have the centromere very close to one end.
- Telocentric chromosomes have the centromere at the tip of the arms.

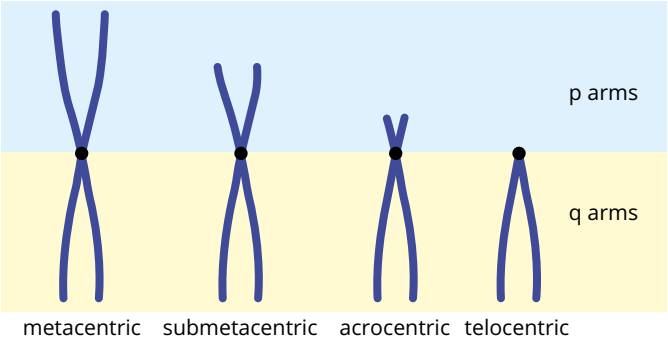


FIGURE 5.1.17 The four major types of chromosomes, named for the position of their centromere: (left to right) meta, submeta, acro and telo.

Variation in number of chromosomes between species

In eukaryotic organisms (plants, fungi, animals), cells that have a nucleus contain a fixed number of chromosomes. The number of chromosomes in somatic cells is characteristic of a species.

The **ploidy** level of a cell is the number of chromosome sets that it carries. Gametes have nuclei that contain only one set of chromosomes and they are called haploid (n). Somatic cells are diploid ($2n$) because they contain two sets of chromosomes: one from each parent. Haploid and diploid cells, and the processes by which they are produced, are covered in detail in Chapter 6.

The diploid chromosome numbers of organisms vary widely, as shown in Table 5.1.2. In humans, the diploid number is 46. In some species of Australian ants, the diploid number is 2: each ant has only one pair of chromosomes ($n = 1$). Some ferns have more than 1000 chromosomes in each somatic cell.

TABLE 5.1.2 Diploid numbers of chromosomes in various species of eukaryotes

Organism	Diploid number ($2n$)
Animals	
horse nematode worm (*Parascaris equorum*)	2
fruit fly (*Drosophila melanogaster*)	8
koala (*Phascolarctos cinereus*)	16
cat (*Felis catus*)	38
human (*Homo sapiens*)	46
chimpanzee (*Pan troglodytes*)	48
platypus (*Ornithorhynchus anatinus*)	52
dingo (*Canis lupus dingo*)	78
Plants and algae	
pipe-cleaner moss (*Ptychomnion aciculare*)	14
garden pea (*Pisum sativum*)	14
cabbage (*Brassica oleracea*)	18
all eucalypts (*Eucalyptus* spp.)	22
blackwood (*Acacia melanoxylon*)	26
pink rock orchid (*Dendrobium kingianum*)	38
single-celled alga (*Euglena gracilis*)	90
coconut palm (*Cocos nucifera*)	596
fern (*Ophioglossum reticulatum*)	1260
Fungi	
mould (*Penicillium* spp.)	2
rust fungus (*Puccinia graminis*)	6
oyster mushroom (*Pleurotus ostreatus*)	22
edible mushroom (*Agaricus bisporus*)	26
brewer's yeast (*Saccharomyces cerevisiae*)	32

Homologous chromosomes

Humans have 46 chromosomes: 23 inherited from their mother and 23 inherited from their father. Forty-four of these chromosomes form 22 matching pairs. The same genes are found at the same locations (loci) on the two chromosomes in a matching pair. They are referred to as homologous chromosomes or homologues.

Figure 5.1.18 shows chromosomes at metaphase. Each pair of chromosomes are said to be homologues because they contain the same gene sets. The sex chromosomes for all females are also homologues because they have a matching pair of X chromosomes. The sex chromosomes for males are not homologous because they have an X and a Y chromosome, which contain different gene sets.

Nevertheless, in most mammals, the X and Y chromosomes behave as a homologous pair during meiosis because some small regions of these chromosomes are homologous.

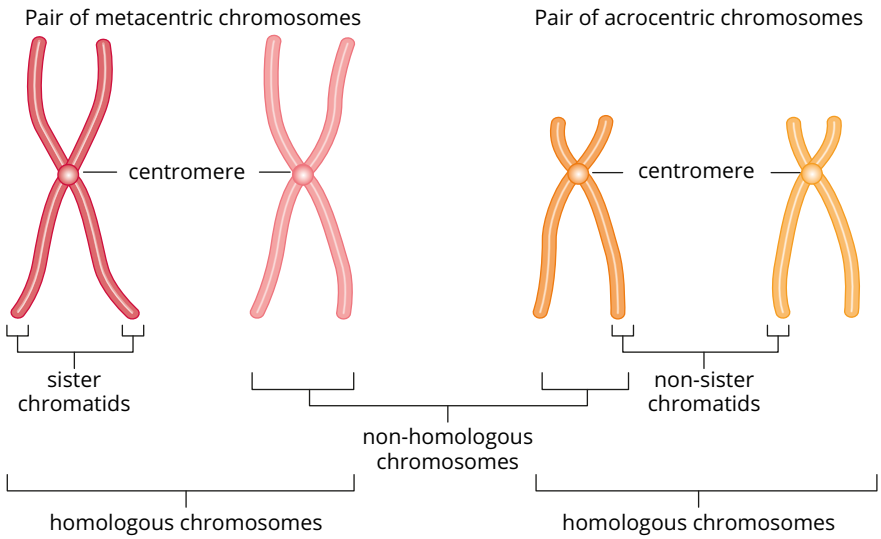


FIGURE 5.1.18 Homologous and non-homologous chromosomes.

In Figure 5.1.19, two human chromosome 7 homologues are shown during metaphase. The loci for three genes are shown: a collagen gene, the cystic fibrosis gene and the Kell gene, which produces a protein involved in determining the Kell blood groups. The three genes would be in this same position on chromosome 7 in all cells in most individuals.

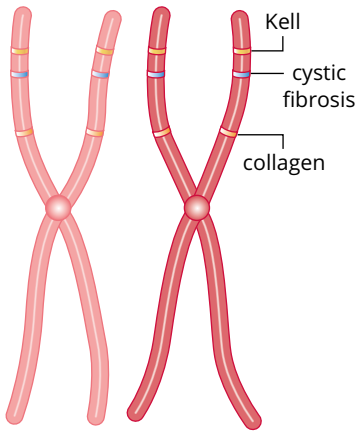


FIGURE 5.1.19 The two human chromosome 7 homologues during metaphase. The locus for three genes is shown: a collagen gene, the cystic fibrosis gene and the Kell gene.

In actively dividing cells, there is a period during the cell cycle, after chromosome replication and before cytokinesis, when there are four copies of each gene in the diploid nucleus, but after cell division there can be no more than two alleles in total (one from each parent). (Cell replication is explained in more detail in Chapter 6.) However, cells are not always actively dividing, and so most of the time chromosomes appear as a single line (Figure 5.1.20). Consequently, non-dividing cells have two copies of each gene, so they have two copies of alleles (which may be identical or different for each gene).

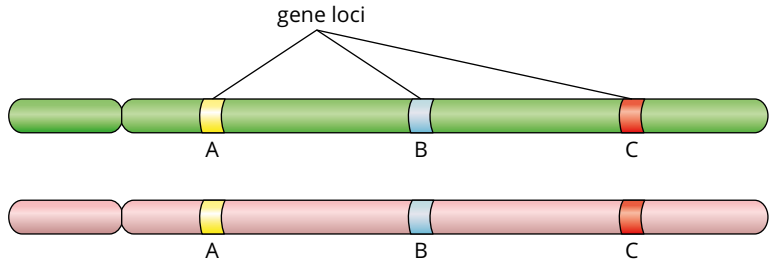


FIGURE 5.1.20 Chromosomes in non-dividing cells consist of one strand. Non-dividing cells have two copies of each chromosome and therefore two copies of each gene.

Sex chromosomes

Sex chromosomes (also called allosomes) are chromosomes involved in sex determination. Chromosomes that are not involved in sex determination are called autosomes. In humans and all other mammals, a pair of chromosomes known as the X and Y chromosomes determine the sex of an individual. Other types of organisms may have different types of sex-determining chromosomes (Table 5.1.3).

TABLE 5.1.3 Examples of sex determination in different organisms

Examples of organisms	Female	Male
humans, other mammals, fruit flies	XX	XY
birds, butterflies, strawberries	ZW	ZZ
grasshoppers, moths	XX	XO
plants	XX	XY

Some organisms (such as fungi and algae) do not have sex-determining chromosomes and therefore do not have sexes; instead they have 'mating types'. Individuals with two similar sex chromosomes are the **homogametic** sex. Individuals with different sex chromosomes are the **heterogametic** sex.

SCIENCE AS A HUMAN ENDEAVOUR

The discovery of DNA

The story of the discovery of the structure of DNA illustrates how our scientific understanding of the world is the result of the communication and sharing of ideas, observations and information to build and test new models. While important 19th-century biologists such as Gregor Mendel and Charles Darwin understood that genetic traits were passed through generations, they did not have knowledge of the underlying molecular basis for genetics or the chemical structures of the molecules of inheritance. Our understanding of genetics and the discovery of DNA has developed since the 1800s and it is now possible to manipulate DNA for use in medicine and agriculture.

In the late 1920s, Frederic Griffith was studying the bacteria that causes pneumonia, *Streptococcus pneumoniae*. He was able to transform a non-disease-causing strain of the bacteria into a pathogenic (disease-causing) strain by mixing the cells from dead pathogenic cells with living non-pathogenic ones. His work clearly demonstrated a change in the genotype and phenotype of the organism. Most importantly, he noticed that this change was heritable.

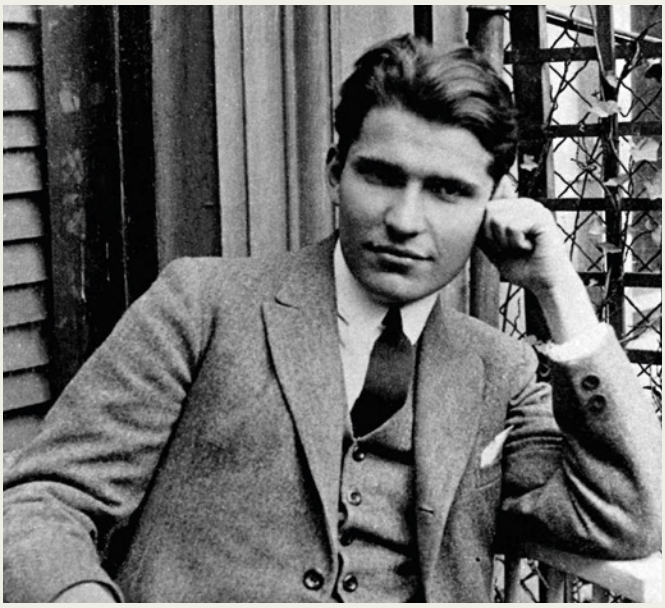


FIGURE 5.1.21 Erwin Chargaff, the biochemist who identified the relative proportions of nucleotide base pairs in DNA.

Further studies by Oswald Avery, Maclyn McCarty and Colin MacLeod in 1944 identified these transforming molecules as deoxyribose nucleic acid (DNA). Initially their work was not well received because so little was known about DNA, and the major theories for the transmission of genetic information had supposed that proteins were the carriers of genetic information. The proposal put forward by Oswald Avery and his team was eventually supported by Alfred Hershey and Martha Chase in 1952, when they used radioactive tags on bacteriophage DNA to trace the passage of genetic information entering host bacteria. This experiment clearly identified DNA as the molecule of inheritance.

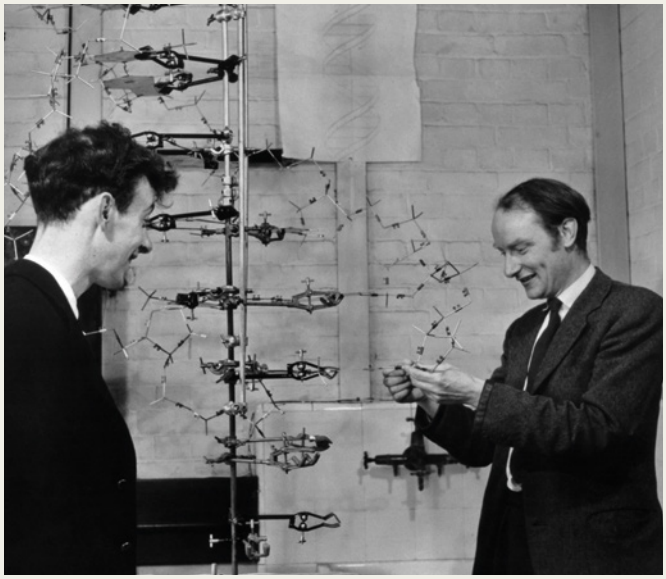


FIGURE 5.1.22 James Watson and Francis Crick with their three-dimensional molecular structure of DNA.

Further evidence for the structure of the DNA molecule came from the work of Erwin Chargaff (Figure 5.1.21). Chargaff reported a distinct regularity in the ratio of nucleotide bases of DNA. The proportion of adenine always matched the proportion of thymine, and the proportion of guanine always matched cytosine. This became known as 'Chargaff's rule,' but the chemical understanding for this observation was not discovered until the three-dimensional (3D) molecular structure of DNA was finally determined by James Watson and Francis Crick in 1953 (Figure 5.1.22).

During the 1950s, many scientists were working to reveal the 3D structure of nucleic acids. Watson and Crick were studying protein st ructures using X-ray crystallography techniques at Cambridge University in the UK. The team of Maurice Wilkins and Rosalind Franklin, working at Kings College in London, had produced images of X-ray diffraction patterns for DNA (Figure 5.1.23 on page 260). These images are produced when X-rays are deflected by molecules as they pass through a purified sample of the substance being studied.

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SCIENCE AS A HUMAN ENDEAVOUR

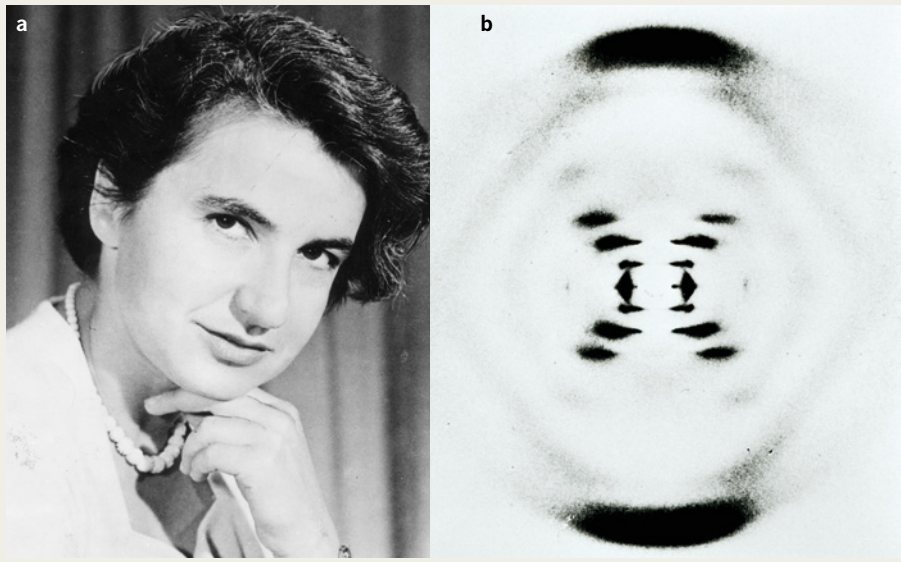

FIGURE 5.1.23 (a) Rosalind Franklin. Her research using (b) X-ray crystallography to elicit complex structures of polymers was crucial to the discovery of the structure of DNA.

The pattern produced by Franklin was consistent with a helical structure, similar to other helical structures that Watson had observed. Franklin's research group had also obtained data suggesting the distances for the width of the helix and spacing for the nitrogenous bases along the sugar–phosphate backbone. It was Franklin who proposed that the DNA molecule's sugar–phosphate backbones were on the outside of the molecule, not on the inside, as Watson, Crick and Linus Pauling had initially suggested. This combination of evidence implied that the DNA molecule was made up of two antiparallel strands, or a double helix.

The 3D model of the DNA structure was constructed by linking the X-ray crystallography images produced by Franklin and Wilkins, showing the position of the sugar–phosphate backbone, with the ratio of the nitrogenous bases given by Chargaff's rule. Watson and Crick published their work in the journal *Nature* in April 1953.

This model for the structure of DNA has become central to our modern understanding of molecular biology and inheritance. Watson, Crick and Wilkins were awarded the Nobel Prize in 1962 for their work. Franklin was not awarded the Nobel Prize because she had died from cancer in 1958, possibly due to her work with X-rays.

Review

1 **a** Identify the key sets of experiments that led to the understanding that DNA was the hereditary material, rather than protein.
   b Consider the evidence Watson and Crick used to correctly describe the structure of DNA.

2 A fly has the following percentages of nucleotides in its DNA: 27.3% A, 27.6% T, 22.5% G and 22.5% C. Explain how these numbers demonstrate Chargaff's rule.

5.1 Review

SUMMARY

- Nucleic acids are polymers of nucleotides (polynucleotides). There are two types of nucleic acids: DNA and RNA.
- Nucleotides are made up of a five-carbon sugar, a phosphate and a nitrogenous base.
- The nitrogenous bases adenine and guanine are purines. The nitrogenous bases cytosine, thymine and uracil are pyrimidines.
- Nucleotides are joined in a condensation polymerisation reaction to form polynucleotides. Water is released and a covalent bond (phosphodiester bond) forms joining the nucleotides together.
- DNA is a long, coiled, double-stranded nucleic acid that forms a double helix.
  - Each strand of DNA contains nucleotides that are made up of deoxyribose sugar, a phosphate and one of four nitrogenous bases (adenine, cytosine, guanine and thymine).
  - The two strands of DNA are joined by complementary base pairing between the nitrogenous bases. Adenine joins with thymine by two weak hydrogen bonds, while cytosine joins with guanine by three hydrogen bonds.
  - The two strands of DNA are antiparallel. One runs in the 5' to 3' direction, while the other runs in the opposite direction.
- RNA is a short, usually single-stranded nucleic acid.
  - RNA contains nucleotides that are made up of ribose sugar, a phosphate and one of four nitrogenous bases (adenine, cytosine, guanine and uracil).
  - There are three main forms of RNA: mRNA, tRNA and rRNA. Each type of RNA plays a particular role in protein synthesis.
- Chromosomes are structures that contain DNA coiled around histones.
- Genes on homologous chromosomes occur at the same loci but may have different alleles.

KEY QUESTIONS

Retrieval

1 Recall the name given to the group of chemical building blocks from which DNA is made.

2 Explain the functions of DNA and RNA.

3 Identify the three basic units of a nucleotide. Illustrate the structure of the nucleotide with a suitable schematic diagram.

Comprehension

4 Explain why a ladder is often used as a metaphor for the structure of DNA. Refer to the specific components of the nucleotide in your response.

5 Explain the meaning of the term 'complementary' with reference to the strands of the DNA molecule. Include a diagram to illustrate your explanation.

6 One strand of DNA has the sequence ATTCCGTA. Write this out. Underneath it, write the sequence of the complementary strand.

7 Copy this table and complete it by assigning 'purine' or 'pyrimidine' to each nitrogenous base and filling in its complementary base.

Base	Purine or pyrimidine structure	Complementary base	Complementary base purine or pyrimidine structure
adenine			
guanine			
cytosine			
thymine			
uracil			

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5.1 Review *continued*

8 Explain how the antiparallel DNA strands ensure the stability of the secondary structure of the molecule.

9 Distinguish between the following terms:

a autosome and allosome

b homologous and non-homologous chromosomes

Analysis

10 Explain the structural differences between prokaryotic and eukaryotic cells and the effect they have on the rate of protein synthesis.

11 Construct a table or diagram to compare the structures of RNA and DNA. Include at least three distinct characteristics.

12 The following table shows the composition of bases in the cells in a variety of species.

Composition of bases in cells of selected species.

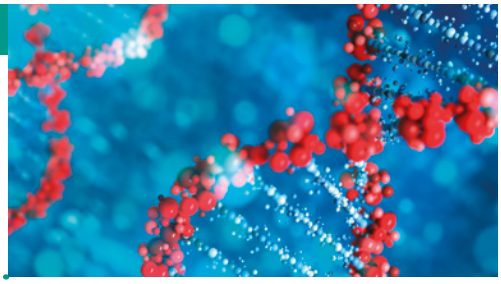
Species	Thymine (%)	Adenine (%)	Guanine (%)	Cytosine (%)
humans	30.1	30.4	19.6	19.9
cattle	28.7	29.0	21.2	21.2
salmon	29.1	29.7	20.8	20.4
wheatgerm	27.4	28.1	21.8	22.7
E. coli	23.6	24.7	26.0	25.7
sea urchin	32.1	32.8	17.7	17.3

Argue whether or not the evidence supports the theory that there are complementary base pairs.

5.2 Genetic expression

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- define the terms genome and gene
- understand that genes include coding and noncoding DNA
- explain the process of protein synthesis in eukaryotes
- recognise that the purpose of gene expression is to synthesise a functional product such as a protein or RNA.



The genetic code represents the genetic information stored in DNA as a triplet code within sections called genes. The **genome** is the complete set of genetic material (DNA) present in an organism, measured in the number of base pairs contained in a haploid set of chromosomes. This information is used to synthesise the amino acid sequences that form proteins through a process called gene expression (Figure 5.2.1).

In this module, you will learn about the roles of DNA and RNA in protein synthesis and the different steps of gene expression.

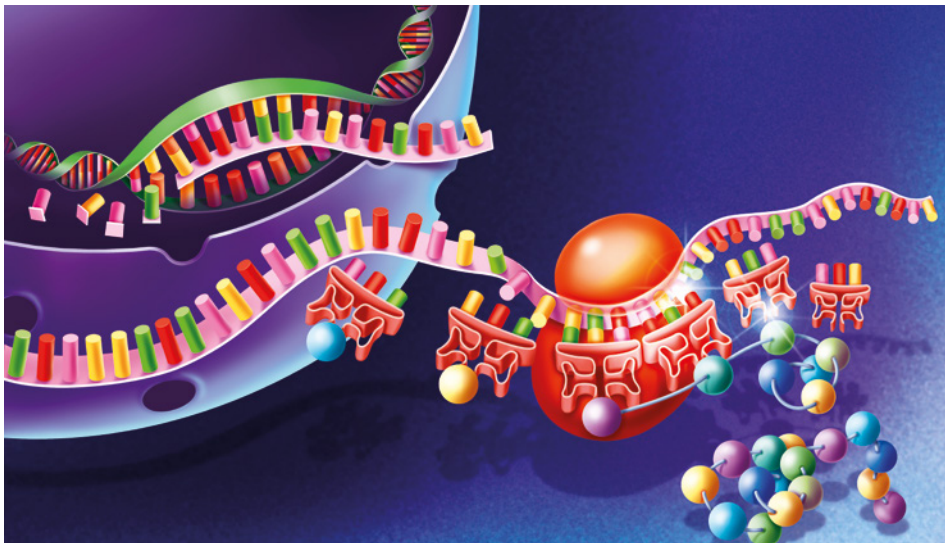


FIGURE 5.2.1 Overview of gene expression, showing messenger RNA being produced during transcription and a polypeptide being synthesised during translation.

GENES, GENOMES AND ALLELES

The somatic cells (all cells in a body except gametes) of most sexually reproducing organisms are **diploid** ($2n$). This is because they contain two sets of chromosomes (one set from each parent). The DNA that makes up these chromosomes ranges in size from 50 million to 300 million base pairs.

The genome of an organism is the total of an organism's DNA measured in the number of base pairs contained in a **haploid** (n) set of chromosomes. The genome is measured in haploid because not all organisms carry a diploid state. Some eukaryotic organisms, such as fungi and algae, are haploids but will produce specialised diploid cells during sexual phases of their lifecycles. The genome of most prokaryotes is contained within one single circular chromosome. Some bacterial genomes consist of several different chromosomes.

A typical human cell has two similar sets of chromosomes and each set has DNA totalling 3234 million base pairs. In other words, the human genome has approximately 3.234×10^9 DNA base pairs.

Genes and genomes

DNA is the molecule of life that encodes the information on which organisms are built (Module 5.1). This information is stored as a sequence of nucleotides that encodes biological information. The order of the nucleotides in this biological code determines which products are synthesised. These products are mainly proteins, but they may also be functional RNA molecules.

A gene is a region of DNA that contains the information to produce a protein or a functional RNA molecule. The fixed position of the gene on the chromosome is called the **locus**. A DNA molecule consists of many genes, and these genes determine the characteristics of an organism. At a molecular level, a gene is a unique sequence of DNA.

Each gene carries a particular instruction, for example, how to make silk for a spider web (Figure 5.2.2). The web is made of silk fibroin, which is a structural protein. The genetic information on how to make this protein is in one particular gene, called the silk fibroin gene. The process by which the information in the gene is decoded to assemble silk fibroin is called the **gene expression**.

Nearly all genes specify the production of a polypeptide chain or protein that performs essential functions in our body's cells. For example, the gene that codes for salivary amylase (which produces sugars from starch) is called the *AMY1* gene and is located on chromosome 1. The protein **haemoglobin** in red blood cells carries oxygen (Figure 5.2.3). It is formed by linking four subunits of polypeptides, two β-globin molecules and two α-globin molecules. The genes that code for the production of functional haemoglobin subunits are called *HB-B*, located on chromosome 11, and *HB-A1*, located on chromosome 16.

Proteins control cellular functions and genes control the production of proteins; therefore, inherited genes ultimately govern the functions of organisms. The length of the sequence of DNA and the precise order of the base pairs in a gene are the critical factors that determine what the gene product will be like and what it will do in a cell.

A gene is a unit of heredity. It is a unique sequence of DNA that determines a characteristic of an organism.

FIGURE 5.2.2 The silk fibroin gene carries instructions for making the silk to create a spider web.

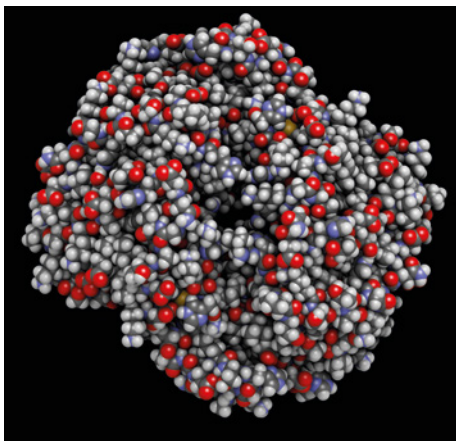


FIGURE 5.2.3 The information found in the genes *HB-B* and *HB-A1* is used to produce haemoglobin, a protein that transports oxygen. around the body in red blood cells.

Alleles

You can see, simply by looking, that there is variation in the human population. Physical characteristics or **traits**, such as skin colour, eye colour and hair colour, all vary within populations and even within families.

Although an individual gene may be responsible for a specific trait, that gene can exist in different forms known as **alleles**. For example, the *TAS2R38* gene that codes for the ability to taste phenylthiocarbamide (PTC) is found on chromosome 7. PTC is a chemical that is found in many common foods and drinks, like coffee, and bitter-tasting vegetables (Figure 5.2.4). PTC is detected by the bitter-taste receptors on the tongue. The shape of the protein receptor determines how strongly PTC will bind to it. The more strongly it binds, the more a person is able to taste the chemical.

Two common alleles of the *TAS2R38* gene exist. One is known as a tasting allele. It codes for a receptor protein that strongly binds PTC. The other is known as a non-tasting allele. It codes for a differently shaped receptor molecule that binds PTC less strongly. This means that the DNA sequence of bases for these two alleles is slightly different. Both alleles still code for a taste receptor protein on the tongue and both are found in the locus on chromosome 7.

Somatic cells of a diploid organism contain two alleles for every gene, with one allele for every gene in an organism inherited from each parent.

An allele is one form of a gene.

The set of alleles present in the DNA of an individual organism is known as the organism's genotype.

GENES AND THE GENETIC CODE

A gene is a region of DNA that may be translated into a polypeptide or an RNA molecule that can be functional, such as tRNA. When coding for a polypeptide, it is the sequence of nucleotides within a gene that contains the information for the protein to be synthesised. Genes can be millions of nucleotides in length. The longest known gene to date, which codes for the protein dystrophin, is 2.5 megabase pairs long (2.5×10^6 base pairs). Some of the shortest genes are fewer than 300 base pairs long.

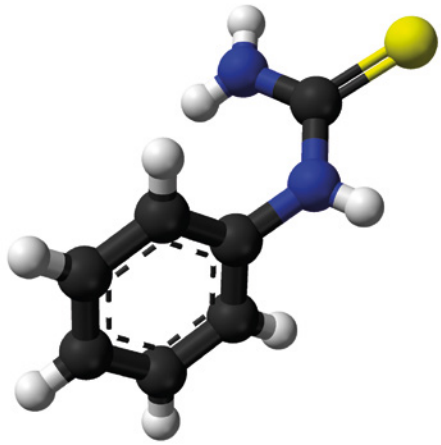


FIGURE 5.2.4 The chemical structure of phenylthiocarbamide (PTC) is similar to other toxins found in poisonous plants.

The genetic code

The **genetic code** is a set of rules that defines how the information in nucleic acids (DNA and RNA) is translated into proteins and functional RNA molecules. The information in DNA and RNA is stored as a three-letter code of nucleotides. In DNA, this three-letter code is called a **triplet**. When a DNA triplet is transcribed into mature mRNA, the triplet is called a **codon**. Each triplet or codon codes for one amino acid (Figure 5.2.5), and may also provide specific instructions, such as 'start translation' and 'stop translation'. These amino acids form polypeptide chains and the polypeptide chains form proteins.

The genetic code is universal—the rules are the same for all organisms on Earth.

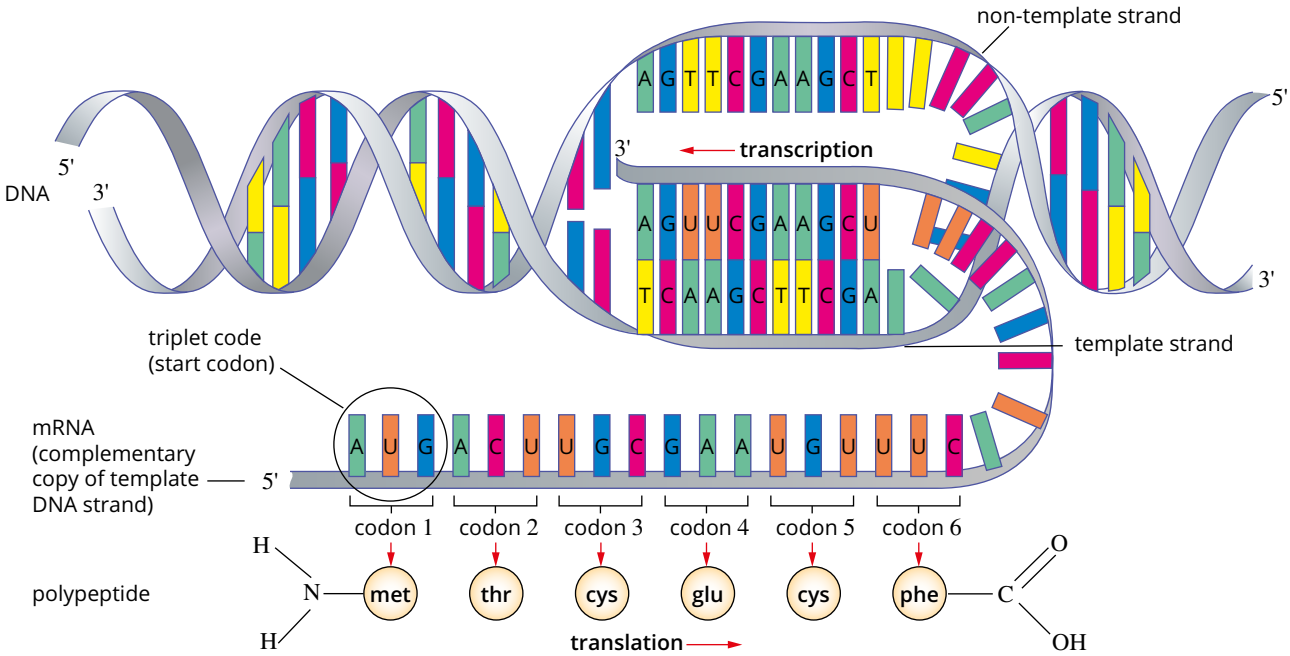


FIGURE 5.2.5 Each codon codes for a particular amino acid.

Messenger RNA is produced during transcription and then translated to produce an amino acid chain (polypeptide).

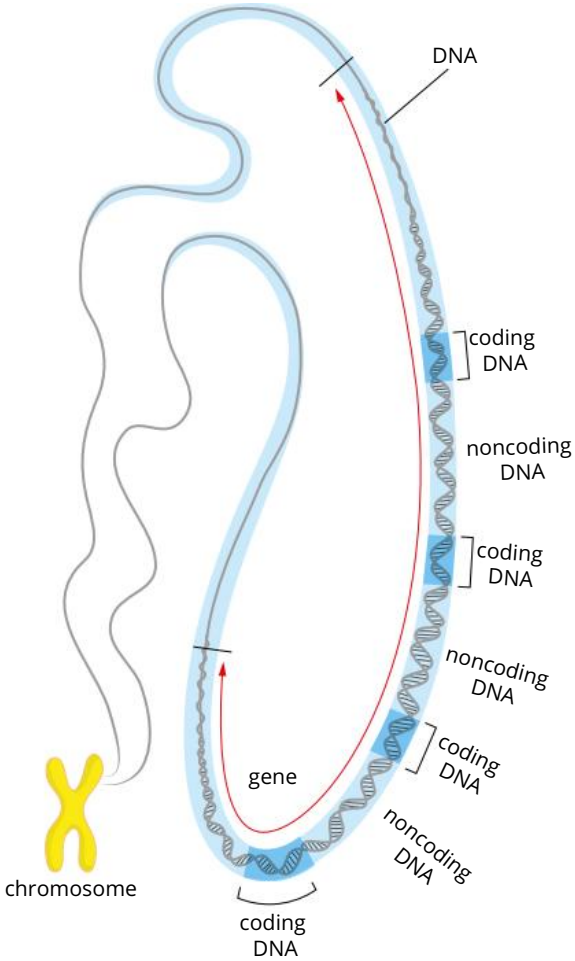


FIGURE 5.2.6 The location of noncoding DNA sequences.

Noncoding DNA

Genes consist of regions of DNA that can either code for amino acids or be **noncoding DNA** sequences. Noncoding DNA sequences are also found between genes (Figure 5.2.6). Noncoding DNA (once called 'junk' DNA) is thought to be very important for **gene regulation** and may control the interaction between DNA, RNA and proteins.

One type of noncoding DNA is located in the centromere region of the chromosome (Figure 5.2.7). This region of noncoding DNA on the chromosome may interact with DNA-binding proteins to regulate the separation of the chromosomes during mitosis. (Mitosis is part of eukaryotic cell replication and will be discussed in Chapter 6.) When chromosome separation is not well regulated, mutations may occur (Module 5.4). Another important form of noncoding DNA found in eukaryotic cells are the telomeres (Figure 5.2.7). These are short repeating nucleotide sequences (in humans, it is TTAGGG). The telomeres protect the DNA molecule from being eroded during cell replication. Without this telomeric DNA, the DNA strands become shorter and shorter with each mitotic division and, if repeated often enough, entire genes could be deleted.

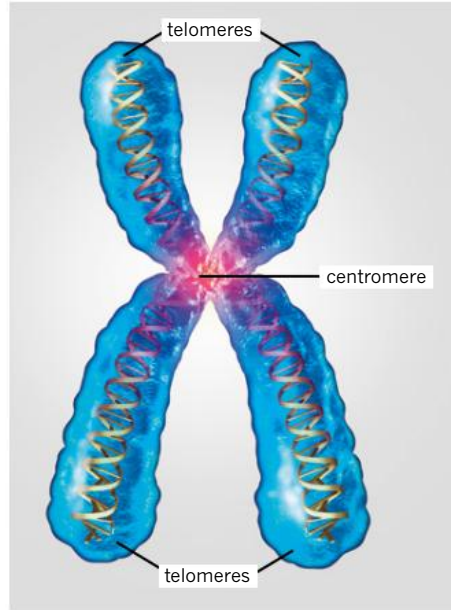


FIGURE 5.2.7 The location of the centromere and telomeres on a chromosome.

Second base of codon

First base of codon	U		C		A		G		Third base of codon
U	UUU	phenylalanine (Phe)	UCU	serine (Ser)	UAU	tyrosine (Tyr)	UGU	cysteine (Cys)	U
	UUC		UCC		UAC		UGC		C
	UUA	leucine (Leu)	UCA		UAA	STOP	UGA	STOP	A
	UUG		UCG		UAG		UGG	tryptophan (Trp)	G
C	CUU	leucine (Leu)	CCU	proline (Pro)	CAU	histidine (His)	CGU	arginine (Arg)	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	glutamine (Gln)	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	isoleucine (Ile)	ACU	threonine (Thr)	AAU	asparagine (Asn)	AGU	serine (Ser)	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	lysine (Lys)	AGA	arginine (Arg)	A
	AUG	methionine (Met) START	ACG		AAG		AGG		G
G	GUU	valine (Val)	GCU	alanine (Ala)	GAU	aspartic acid (Asp)	GGU	glycine (Gly)	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	glutamic acid (Glu)	GGA		A
	GUG		GCG		GAG		GGG		G

FIGURE 5.2.8 The genetic code for the 20 amino acids and stop codons.

Degeneracy

The genetic code is said to be **degenerate** because more than one codon can code for the same amino acid (Figure 5.2.8). Differences in codons encoding the same amino acid usually occur at the second or third base. As the genetic code uses four nucleotides and three nucleotides to code for an amino acid, there are 64 possible codons ($4^3 = 64$) available to code for the total 20 amino acids (Figure 5.2.8).

The structure of a gene

All eukaryotic genes have a number of structural features in common (Figure 5.2.9):

- start and stop triplets
- promoter regions
- exons
- introns.

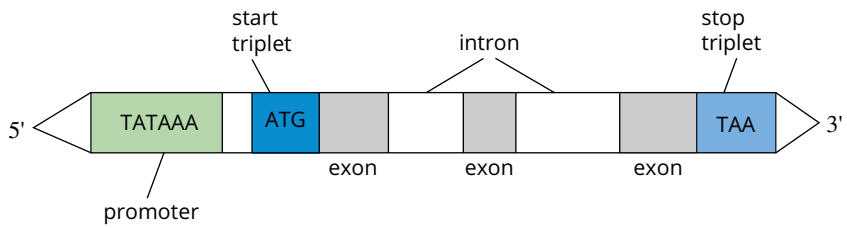


FIGURE 5.2.9 Eukaryotic genes have start and stop triplets, promoter regions, coding exons and noncoding introns.

Start and stop triplets

A start triplet indicates where the first stage of gene expression will begin. When transcribed into mRNA, the DNA triplet ATG becomes the start codon AUG. AUG is the most common start codon in mRNA. The start codon initiates **translation** and codes for the amino acid methionine. Most functional proteins start with methionine, but there are rare exceptions. For example, a protein in the fungus *Candida albicans* uses GUG as a start codon.

A stop triplet indicates where transcription will end. The stop triplet does not code for an amino acid. When stop triplets are transcribed into mRNA, they become the codons UAA, UAG and UGA.

Promoter region

Promoter regions are sections of a gene that are found before the start triplet, at the 5' end of the site where transcription will begin. A promoter region:

- is the location where the **RNA polymerase** (the enzyme that initiates transcription) attaches to the gene
- identifies which DNA strand will be transcribed
- identifies where transcription of the gene will start
- identifies in which direction transcription will occur.

In many eukaryotic genes, the promoter region is coded for by the sequence of bases TATAAA, which is sometimes called the **TATA box**.

Exons and introns

Exons are regions of a gene that are usually expressed as proteins or RNA. Exons come together to make up mRNA, which is then translated into proteins.

In eukaryotes, not all sections of a gene are translated. **Introns** (or spacer DNA) are noncoding, or intervening, regions of a gene. Introns are spliced out of the mRNA during the stage of gene expression called RNA processing. Many functions of these noncoding sections of DNA are yet to be determined.

There are no rules about the number of exons and introns in a gene. For example, 99% of the length of the dystrophin gene is made up of introns, but the gene for the protein insulin consists of three exons and two introns.

GENE EXPRESSION

Gene expression is the process by which the information stored in a gene is used to synthesise a functional gene product (protein or RNA) (Figure 5.2.10). This process is highly regulated, so that proteins or RNA molecules are only produced if and when they are required by a cell. Multicellular organisms, in particular, can have specialised cells that require a specific set of proteins. For example, in humans, the cells in connective tissue and bone require the protein fibrillin to form elastic fibres, and skin cells require the enzyme tyrosinase to produce melanin and other pigments. The ability to regulate gene expression conserves energy and materials (nucleotides and amino acids) in the cell.

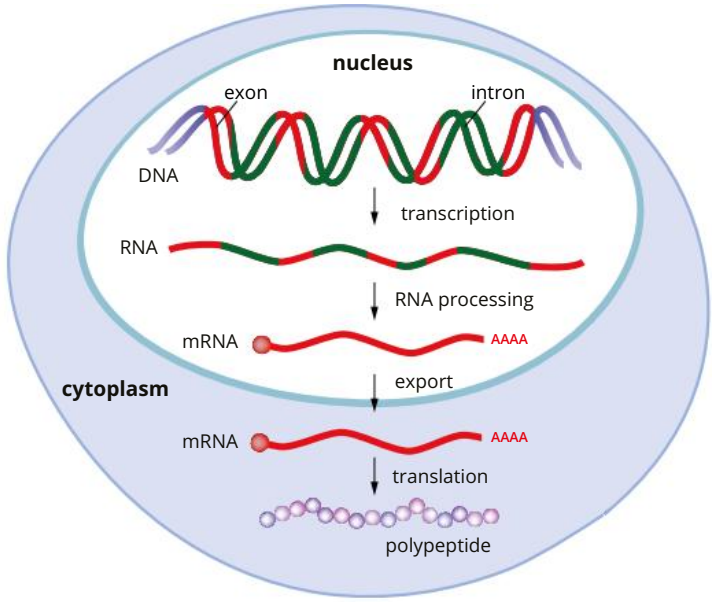


FIGURE 5.2.10 The information stored in a gene is used to synthesise a polypeptide, which will become a protein.

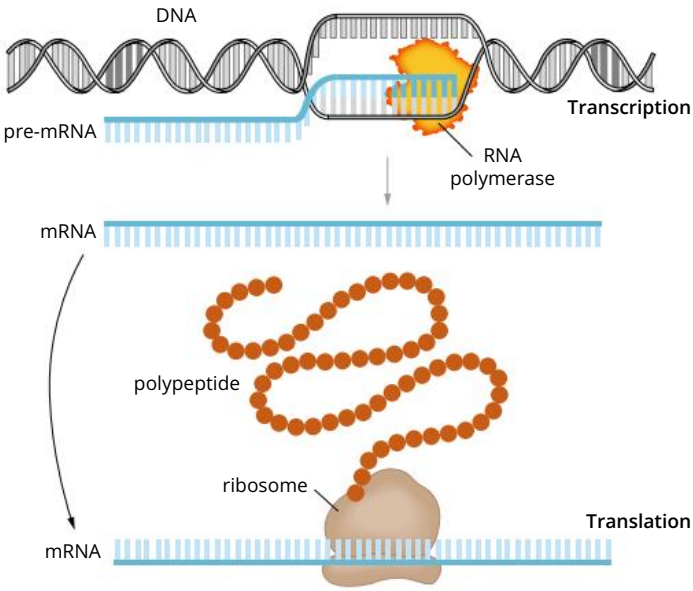


FIGURE 5.2.11 Messenger RNA is formed by transcription inside the nucleus. Mature mRNA then travels outside the nucleus to a ribosome, where translation occurs.

DNA and RNA both play vital roles in protein synthesis. DNA provides the instructions that are translated by RNA into proteins that carry out all of the functions that are essential to life. RNA plays an important role in expressing the information contained in the sequence of a gene to synthesise proteins. Each different type of RNA has a specific role in the process of protein synthesis.

Messenger RNA is formed in the nucleus by the process of transcription. It carries a copy of the nucleotide sequence of DNA that specifies the amino acid sequence for a particular protein. During transcription, pre-mRNA is first formed by the enzyme RNA polymerase. Pre-mRNA is then processed (post-transcriptional modification) to form mature mRNA, which is a single-stranded copy of the **coding DNA** (or gene). The mature mRNA travels from the nucleus to the cytosol where it binds to ribosomes ready for translation (Figure 5.2.11).

Ribosomal RNA is synthesised in the nucleolus of the cell nucleus and is based on the nucleotide sequence of the DNA. Together with proteins, rRNA forms a small organelle called a ribosome. Ribosomes are the sites where the information in the mRNA is translated into a chain of amino acids (Figure 5.2.12).

Transfer RNA molecules transfer amino acids from the cytoplasm to the ribosomes, where they are joined to form a polypeptide chain based on the arrangement of nucleotides in the mRNA. There are 61 different tRNA molecules, each of which combines with only one particular amino acid at one end of its molecule. (There are no tRNA molecules that recognise the three stop codons, which is how translation is terminated.)

There are three places for tRNA to bind to the ribosome: the exit site (E), the peptidyl site (P) and the aminoacyl (A), as shown in Figure 5.2.13. At the other end of the tRNA molecule, there is a sequence of nucleotides known as the **anticodon**. The anticodon recognises a particular sequence of nucleotides in the mRNA. This enables an amino acid to be positioned in the correct space on a protein.

Gene expression leading to protein synthesis in eukaryotic cells occurs in three stages:

- transcription
- RNA processing
- translation.

Transcription—copying the genetic code

The production of single-stranded mRNA from DNA is called **transcription** and occurs within the nucleus of eukaryotic cells. Transcription occurs in three steps: initiation, elongation and termination. The DNA segment that undergoes transcription is known as the transcription unit.

Initiation

Transcription factors are proteins that bind to DNA sequences close to the promoter region of a gene. In eukaryotic cells, transcription factors are required for RNA polymerase to attach to the DNA. RNA polymerase then attaches to the promoter, unwinding and unzipping the DNA molecule by breaking the weak hydrogen bonds between the two strands to expose the bases (Figure 5.2.14a on page 270).

Elongation

During transcription, the RNA polymerase molecule covers a region of approximately 30 base pairs. Within this region, a segment of about 15 base pairs is uncoiled. This forms a transcription bubble. As the RNA polymerase moves along the gene, DNA strands behind the transcription bubble are coiled again. The RNA polymerase moves along the DNA molecule, producing a strand of mRNA. It uses a strand of DNA as a template, attaching nucleotides (A, U, G, C) by complementary base pairing.

Messenger RNA is always synthesised in the 5' to 3' direction, with new nucleotides added to the 3' end. The initial mRNA molecule transcribed is called a primary RNA transcript (Figure 5.2.14b on page 270). The primary RNA transcript is then processed into mature mRNA.

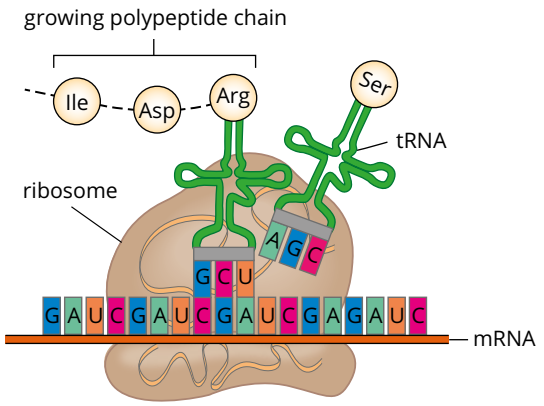


FIGURE 5.2.12 Ribosomal RNA and proteins form a small organelle called a ribosome.

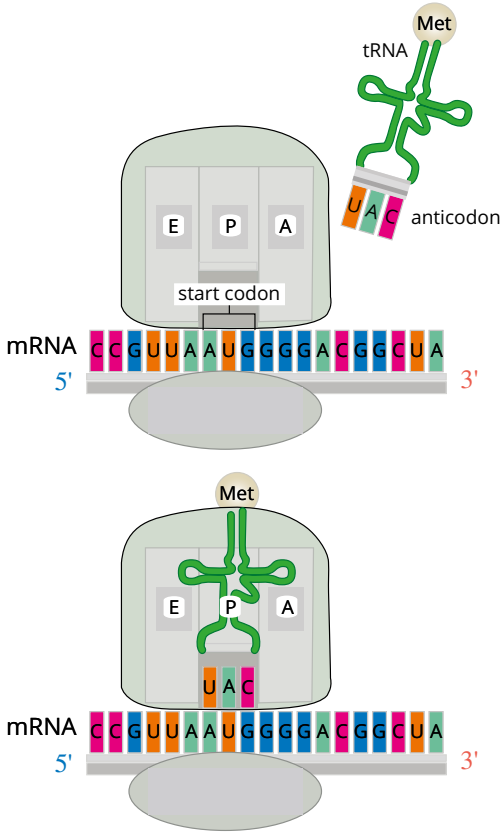


FIGURE 5.2.13 Transfer RNA molecules carry amino acids from the cytoplasm and bind them to a specific spot on the ribosome : the exit site (E), the peptidyl site (P) or the aminoacyl (A).

Transcription is the synthesis of messenger RNA from a DNA template.

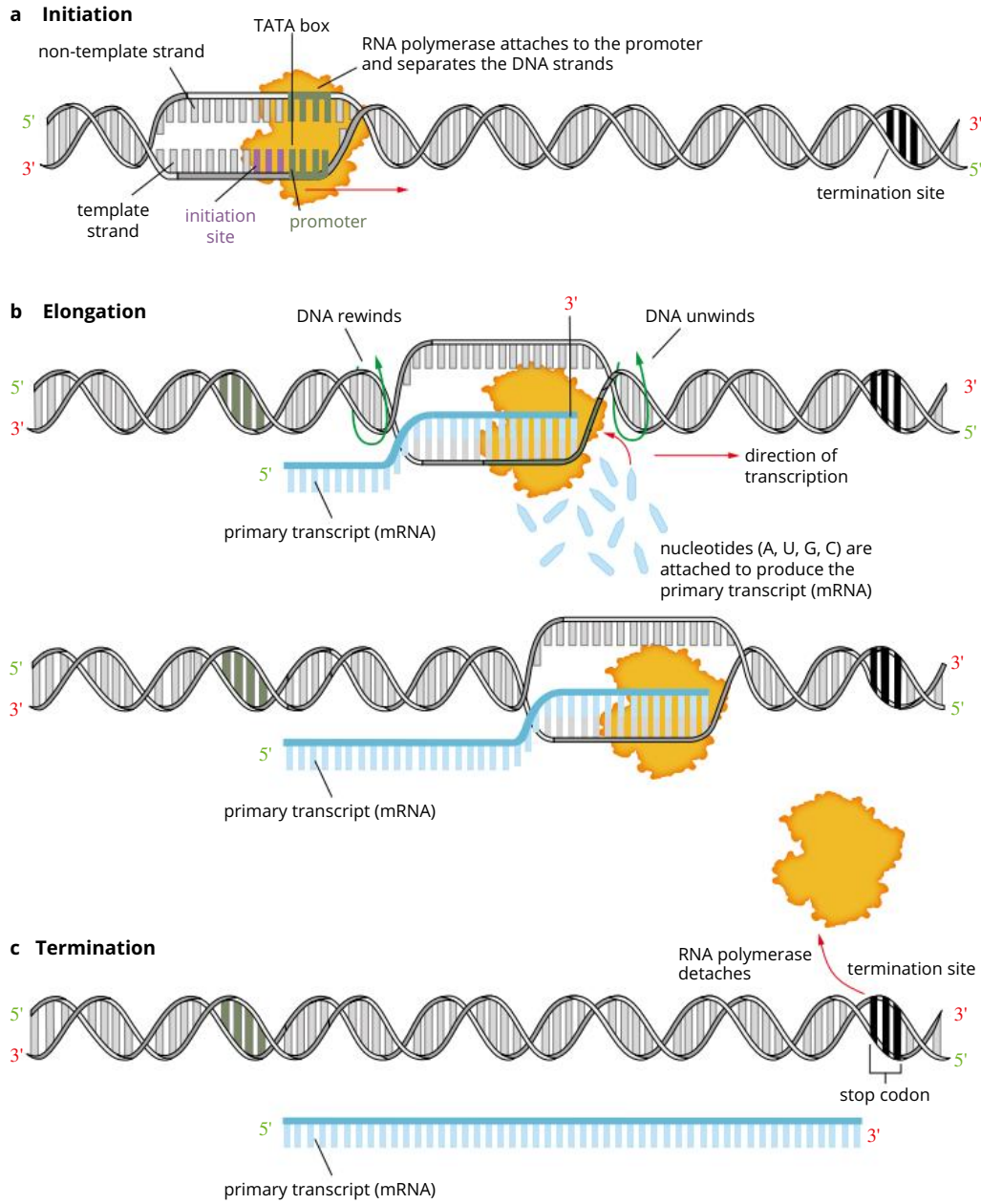


FIGURE 5.2.14 Transcription occurs in three stages: (a) initiation, (b) elongation and (c) termination.

Termination

Transcription ends when RNA polymerase reaches the termination site of the gene. This region contains a stop triplet code, which binds release factors that signal termination. The RNA polymerase detaches, releasing the mRNA and allowing the DNA molecule to reform (Figure 5.2.14c).

Many RNA polymerase molecules may attach to the gene being transcribed, producing many of the same mRNA molecules. The strand of DNA that is transcribed to the mRNA is known as the **template strand**, and the other complementary strand is known as the **coding strand**. The mRNA carries the same base sequence as the coding strand, except it contains uracil in place of thymine.

RNA processing

After transcription, the primary RNA transcript undergoes processing before it is translated. This stage of gene expression is called **RNA processing** and includes:

- the addition of a 5' cap
- the addition of a poly-A tail
- splicing (removal) of the introns (mRNA maturation).

5' cap and poly-A tail

A cap consisting of a methylguanosine triphosphate molecule, called a **5' cap**, is added to the 5' end of the primary RNA transcript while it is being synthesised during transcription. Once transcription has finished, a chain of up to 250 adenine nucleotides is added to the 3' end of the primary RNA transcript. This chain is called a **poly-A tail**.

These modifications to either end of the primary RNA transcript increase its stability and prevent it from degrading. The 5' cap also helps bind the ribosome to the mRNA at the beginning of translation.

Splicing

Eukaryotic genes have protein-coding regions called exons and non-protein-coding regions called introns. Most prokaryotes contain only exons and therefore the RNA processing described in this module does not occur in prokaryotes.

In eukaryotes, before a protein can be produced, the introns must be cut out of the primary RNA transcript to form the mature mRNA molecule. This process is known as **splicing**. During splicing, a complex molecule composed of protein and RNA molecules, called a **spliceosome**, removes the introns from the primary RNA transcript and joins the exon sections together to make mature mRNA (Figure 5.2.15). (Not all of the exons will necessarily be included, as you will learn in Module 5.3.) The single-stranded mature mRNA molecule then exits the nucleus via a nuclear pore.

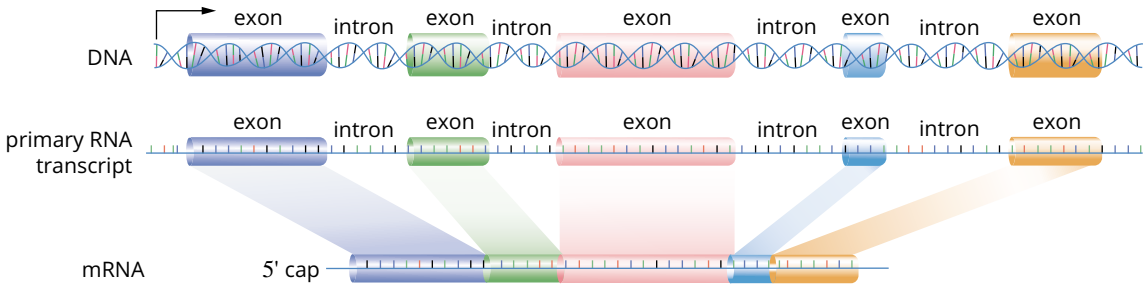


FIGURE 5.2.15 During RNA processing, the introns are spliced from the primary RNA transcript, resulting in mature messenger RNA.

Translation—assembling the protein

Translation is the process in which the codons on mRNA are translated into a sequence of amino acids, resulting in a polypeptide. This process occurs on ribosomes. Ribosomes bind to an mRNA molecule and act as docking stations for the tRNAs to deposit their specific amino acids. A part of the tRNA, called an anticodon, recognises and binds to the codon on the mRNA by complementary base pairing. Each tRNA carries a specific amino acid related to the codon to which it binds.

Translation occurs in a series of steps: initiation, elongation and termination.

Initiation

To begin protein synthesis, a small ribosomal subunit attaches to the 5' end of an mRNA strand. It moves along the mRNA until it reaches a start codon (AUG). The sequence AUG, which codes for the amino acid methionine, is the most common initiation triplet of mRNA (there are some rare exceptions).

A tRNA molecule with the anticodon UAC then brings the methionine to the mRNA. The tRNA molecule joins to the mRNA start codon, attaching by complementary base pairing between the codon and anticodon (Figure 5.2.13). A large ribosomal subunit also attaches to the tRNA and the small ribosomal subunit.

The binding of both ribosomal subunits causes the formation of three special sites for tRNA to bind: the aminoacyl site (A site), the peptidyl site (T site) and the exit site (E site). The attachment of amino acids to their corresponding tRNA molecules occurs in the cytosol—a process that is catalysed by enzymes.

Elongation

Following the attachment of methionine, another tRNA with a complementary anticodon to the next codon on the mRNA attaches and adds its specific amino acid to the growing polypeptide chain (Figure 5.2.16). The deposited amino acid joins by a peptide bond to the first amino acid through a condensation polymerisation reaction. The ribosome then releases the tRNA and moves further along the mRNA strand. At each codon, a new tRNA binds and adds another amino acid. The tRNA molecules can be reused, allowing them to pick up more of their specific amino acids and return to the mRNA molecule.

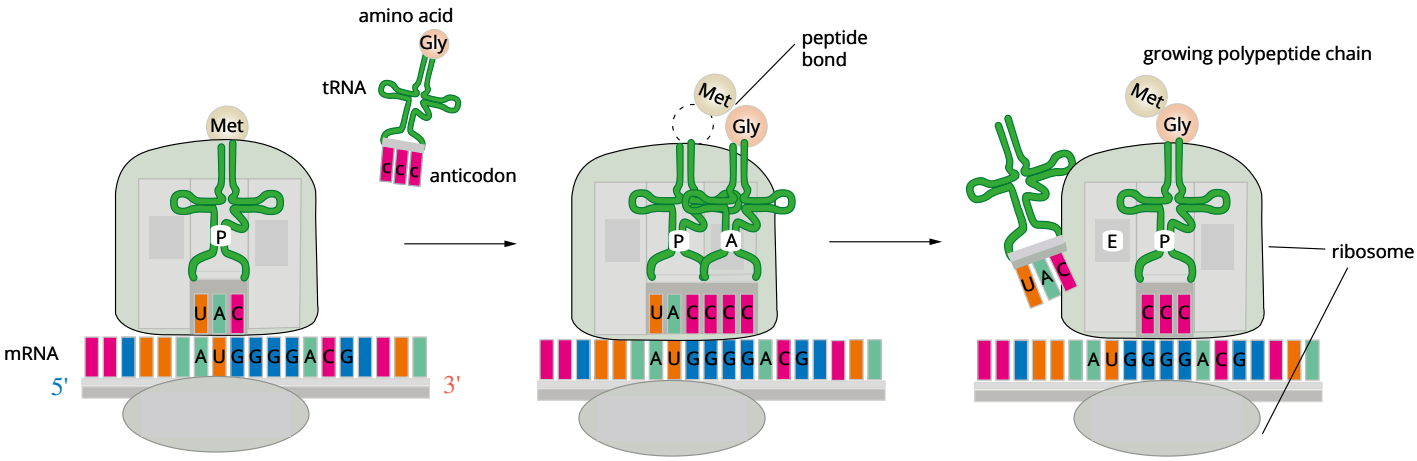


FIGURE 5.2.16 The elongation step in the translation process.

Termination

Attachment of amino acids continues until a stop codon is reached. The polypeptide chain is then released from the ribosome into the cytoplasm or the endoplasmic reticulum. Some proteins consist of more than one polypeptide. The polypeptides of these proteins associate in the cytoplasm or the Golgi apparatus to form the fully functional protein.

Many ribosomes can translate the same, single strand of mRNA, enabling many polypeptide chains to be produced at the same time. Once the polypeptides are fully functional, they either remain in the cell for use, or are exported from the cell via exocytosis for use elsewhere in the organism.

Protein synthesis in eukaryotes

To summarise, protein synthesis in eukaryotes consists of two main stages: transcription and translation (Figure 5.2.17). The first stage is transcription and occurs in the nucleus. The section of the DNA that contains the base sequence for the gene is transcribed (copied) into mRNA. When the mRNA is produced, it leaves the nucleus and enters the cytoplasm. The second stage of protein synthesis, translation, then occurs in the cytoplasm. Ribosomes in the cytoplasm read the nucleotide sequence on the mRNA in groups of three (known as codons) and specific amino acids are brought together to form a polypeptide chain. Eventually the polypeptide chain is folded or modified to form a fully functioning protein.

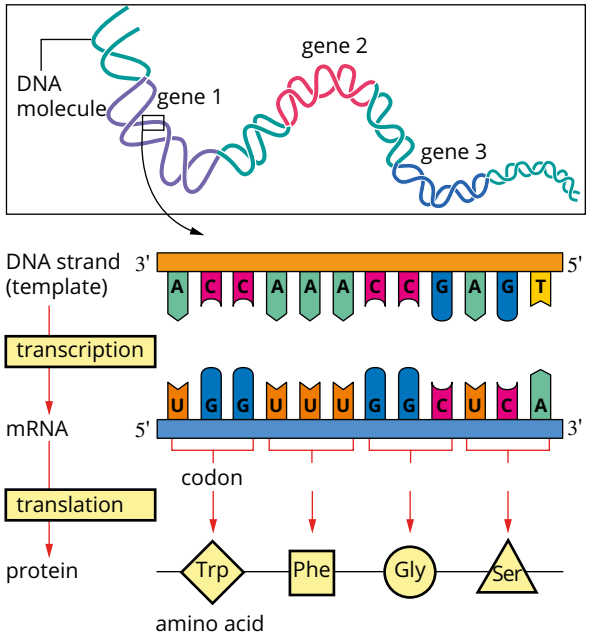


FIGURE 5.2.17 Summary of protein synthesis: transcription and translation.

Protein synthesis in prokaryotes

Prokaryotes do not have membrane-bound organelles, so all cellular processes occur within the cytosol. This allows transcription and translation to be a continuous process rather than two separate stages. Ribosomes are able to attach to the mRNA while it is being transcribed, so translation can occur at the same time. Prokaryotes only contain exons, so splicing is not necessary prior to translation.

Figure 5.2.18 illustrates the many differences between protein synthesis in prokaryotic and eukaryotic cells. These differences have been used to develop drugs that target protein synthesis in prokaryotes only. For example, some antibiotics disrupt or inhibit the production of proteins.

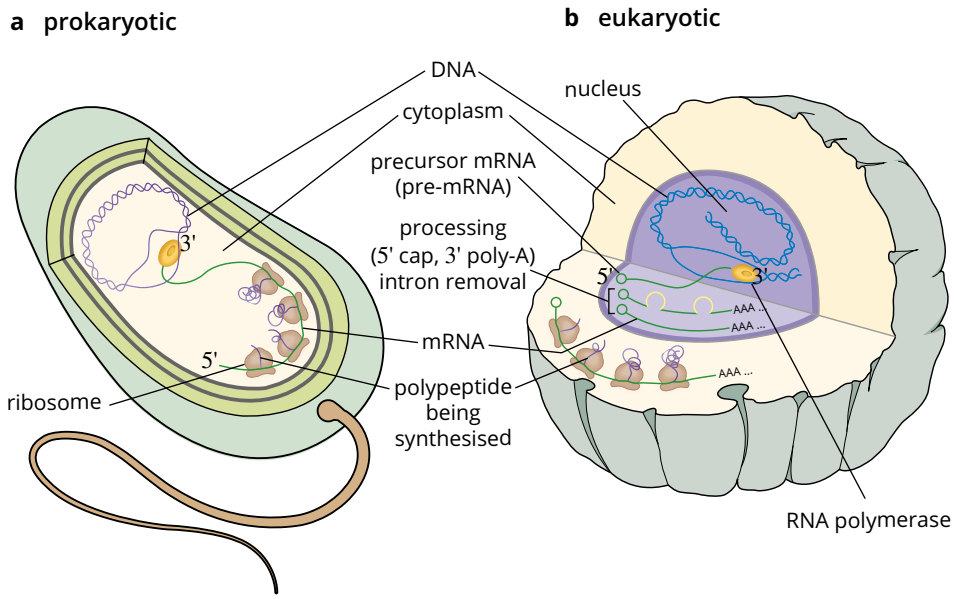


FIGURE 5.2.18 Comparison of protein synthesis in (a) prokaryotic and (b) eukaryotic cells.

WS 4.1.3

5.2 Review

SUMMARY

- The genetic code is the set of rules about how the instructions carried in nucleic acids are translated to synthesise proteins and functional RNA molecules.
- In DNA, this information is stored as a three-letter code of nucleotides known as a triplet. When these triplets are transcribed into mature mRNA, they are then known as codons.
- The genetic code is universal and degenerate. There are 64 possible codons of three nucleotides (e.g. UAC) for the 20 amino acids.
- Eukaryotic genes have a number of structural features in common:
  - stop and start triplets—the three-letter codes that indicate where transcription starts and stops
  - promoter regions—the sites at which the RNA polymerase attaches to the gene to begin transcription (sometimes called the TATA box)
  - exons—the DNA regions that are coding segments
  - introns—the DNA regions that are noncoding segments.
- Gene expression is the process in which the information stored in a gene is used to synthesise a functional gene product (protein or RNA). Gene expression is regulated so that it occurs if and when the particular protein or RNA is required by the cell.
- Protein synthesis in eukaryotes occurs in three stages: transcription, RNA processing and translation.
  - Transcription occurs in the nucleus and involves RNA polymerase transcribing the DNA into a primary RNA transcript.
  - During RNA processing in eukaryotes, a 5' cap is added to the 5' end of the primary RNA transcript and a poly-A tail is added to the 3' end. The cap and tail make the mRNA more stable and prevent it from degrading. Next, the primary RNA transcript is spliced to remove the introns, and sometimes some exons, resulting in mature mRNA. The mature mRNA then exits the nucleus.
  - Translation occurs on a ribosome. The codons on mRNA are translated into a sequence of amino acids, which are delivered by their specific tRNA molecules to form a polypeptide chain.

KEY QUESTIONS

Retrieval

1 Define the following terms:
- **a** DNA
- **b** genome
- **c** gene
- **d** allele

2 Describe these structural features of eukaryotic genes and their functions:
- **a** stop and start codons
- **b** promoter regions
- **c** exons
- **d** introns

3 Describe the difference between coding and noncoding DNA.

4 Name the three stages of protein synthesis.

Comprehension

5 Identify the complementary nucleotides and indicate the 5' and 3' ends of each nucleotide sequence. Assume no RNA processing occurs.

Noncoding strand DNA: 5'-A T G T A T G C C A A T C G A 3'
Coding strand DNA: _-T _____ ___-__'
mRNA: _'-A_____ __-'
Anticodons on tRNA: _'-__ __/__ __/__ __/__ __/__ __/__-__'

6 **a** Identify the correct order for the transcription process. Rearrange the table to match the transcription event with the correct stage at which it occurs.

Transcription process

Stage of transcription	Transcription event
initiation	• RNA polymerase reaches the termination site of the gene (stop codon) and translation ends. • The RNA polymerase moves along the DNA molecule, producing a strand of mRNA. • Transcription factors combine with the region at the start of the gene, known as the promoter. • The RNA polymerase detaches, releasing the mRNA and allowing the DNA molecule to re-form. • RNA polymerase uses a strand of DNA as a template, attaching nucleotides (A, U, G, C) by complementary base pairing. • RNA polymerase attaches to the promoter, unwinding and unzipping the DNA molecule by breaking the weak hydrogen bonds between the two strands to expose the bases.
elongation	
termination	

b Identify the correct order for the translation process. Rearrange the table to match the translation event with the correct stage at which it occurs.

Translation process

Stage of translation	Translation event
initiation	• The tRNA reaches a stop codon. • The polypeptide chain is released from the ribosome into the cytoplasm or the endoplasmic reticulum. • A small ribosomal subunit attaches to the 5' end of an mRNA strand. It then moves along the mRNA until it reaches a start codon (AUG). • The ribosome then releases the tRNA and moves further along the mRNA strand. At each codon a new tRNA binds and adds another amino acid. • Following the attachment of the amino acid methionine, another tRNA, with a complementary anticodon to the next codon on the mRNA, attaches and adds its specific amino acid to the growing polypeptide chain. • A tRNA molecule with an anticodon (UAC) brings the amino acid methionine to the mRNA. The tRNA molecule joins to the mRNA start codon, attaching by complementary base pairing between the codon and anticodon.
elongation	
termination	

continued over page

5.2 Review *continued*

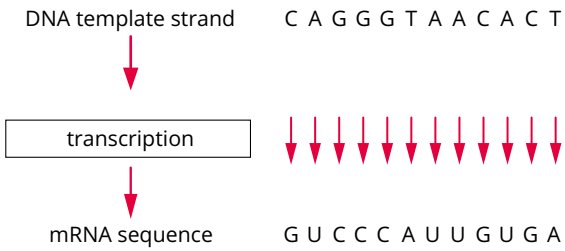
Analysis

To answer questions 7–9, refer to the genetic code in the table below. With a few rare exceptions, the genetic code is accepted as being universal.

Genetic code translation table

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

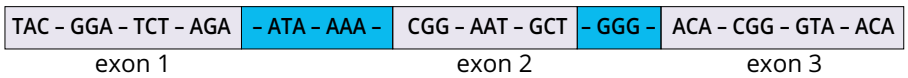
7 The following flow chart represents transcription.



Outline the significance of the fourth codon.

8 The DNA sequence of a particular gene is shown below.

Determine the functional amino acid sequence coded for by this segment of DNA, showing the mature mRNA strand.



9 The DNA sequence of the promoter and first exon of a gene are shown below.

GGGCTCTATAAAGGGTACCACTTCAATGCT

a Determine the amino acid sequence coded for by this DNA template and provide reasoning.

b Suggest the likely consequence for the organism if a mutation changes the sequence of the TATA box.

10 a A sample of double-stranded DNA is obtained and the mRNA from this DNA is transcribed. The base composition of each of the two DNA stands and the mRNA stand is analysed and the results are provided below. The numbers indicate the percentage of each base in the strand:

Base composition of DNA and mRNA from sample 1

	A	G	C	T	U
strand 1	40.1	28.9	9.9	0.0	21.1
strand 2	21.5	9.5	29.9	39.1	0.0
strand 3	40.0	29.0	97	21.3	0.0

i Identify which of these strands is mRNA.

ii Justify which strand is the template strand for the mRNA.

b In a new experiment, a second sample of double-stranded DNA was obtained, the mRNA was transcribed and the results were analysed and presented in the following table. The numbers indicate the percentage of each base in the stand.

Base composition of DNA and mRNA from sample 2

	A	G	C	T	U
strand 1	29.1	39.9	31.0	0.0	0.0
strand 2	0.0	30.0	39.8	30.2	0.0
strand 3	29.4	39.4	31.2	0.0	0.0

Argue the identification of the DNA template and mRNA strand.

5.3 Gene regulation

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- identify factors that regulate the expression of genes
- distinguish between structural and regulatory genes
- recall examples of regulatory transcription factors.

The human genome consists of many thousands of genes. A cell is able to express a selection of these genes at a given time. The genes expressed determine which proteins are produced, giving the cell its functionality and characteristics. Gene expression is the process through which information from a gene is used to synthesise a functional gene product (a protein or RNA). Gene expression in eukaryotes is tightly regulated by multiple mechanisms, at different points. The most important differences in gene regulation between prokaryotes and eukaryotes are due to the lack of cell compartmentalisation in the prokaryote cell.

Although gene expression is controlled at many points, this module focuses on gene regulation at the transcription stage. You will learn the difference between structural and regulatory genes, and understand how some proteins called transcription factors are able to regulate transcription by 'switching genes on and off' (Figure 5.3.1).

FIGURE 5.3.1 Transcription repressor protein (pink) bound to DNA (red and blue). The repressor protein physically blocks access to the DNA, preventing transcription of the underlying gene.

GENE REGULATION IN EUKARYOTES AND PROKARYOTES

Gene regulation is tightly controlled in both eukaryotes and prokaryotes. However, as the process of gene expression in eukaryotes is more complex, gene regulation occurs in a greater number of stages in eukaryotes than in prokaryotes.

Gene expression in prokaryotic cells consists only of transcription and translation, and occurs in the cytoplasm of cells. This is because prokaryotic cells do not have a nucleus or any other membrane-bound organelles. In the absence of a nucleus, the prokaryote can simultaneously transcribe and translate the same gene and the newly made protein can quickly diffuse to its site of function (Figure 5.3.2). The translation of mRNA can begin as soon as the leading 5' end of the mRNA molecule peels away from the DNA template. Gene regulation in prokaryotes only occurs during transcription.

In contrast, eukaryotic cells have compartmentalised organelles, such as the nucleus, which separates transcription and translation into different parts of the cell. In eukaryotic cells, transcription and RNA processing occur within the nucleus and translation occurs in the cytoplasm (Figure 5.3.2). Gene expression in eukaryotes can occur during the processes of transcription, RNA processing or translation. It is highly controlled, and can be regulated at any of these stages.

> **In prokaryotes, gene expression consists only of transcription and translation. In eukaryotes, it involves transcription, RNA processing and translation.**

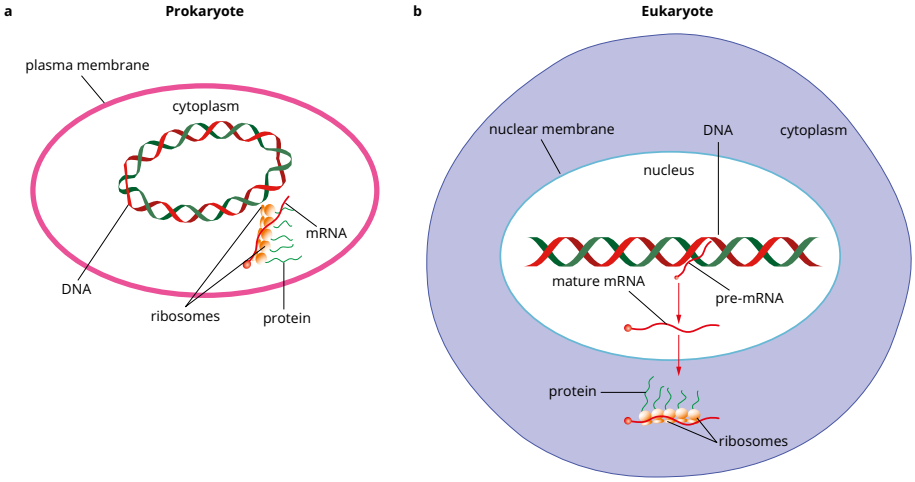


FIGURE 5.3.2 (a) In prokaryotic cells, transcription and translation occur simultaneously in the cytoplasm. (b) In eukaryotic cells, transcription and RNA processing occur in the nucleus and translation occurs in the cytoplasm.

STRUCTURAL AND REGULATORY GENES

Constitutive genes are always switched on, so they are expressed constitutively (continually). For other genes, transcription may be **induced** or **repressed** by transcription factors as needed, depending on the cell type, stage or the specific environmental conditions.

Structural genes code for proteins and RNAs that are not involved in gene regulation. For example, they can code for enzymes, protein channels, protein components for the cytoplasmic skeleton or tRNA, among others.

Regulatory genes code for transcription factors. Transcription factors are proteins that control gene expression at the transcription stage. They bind to DNA sequences close to the promoter region of a gene or to the RNA polymerase to induce or repress the expression of specific genes. (Figure 5.3.3).

Structural genes code for RNA and proteins that are not involved in gene regulation. Regulatory genes control the expression of structural genes via the production of transcription factors.

Genes may be switched on all the time (constitutively expressed) or may be induced or repressed as needed by transcription factors.

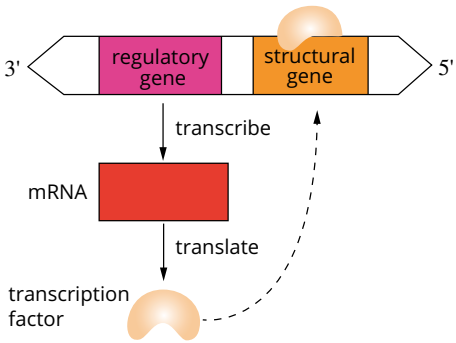


FIGURE 5.3.3 Regulatory genes code for transcription factors that induce or repress structural genes.

The *lac* operon

Gene regulation in eukaryotes is quite complex. To illustrate how transcription factors regulate gene transcription, a simple prokaryotic model, the ***lac* operon** is commonly used (Figure 5.3.4).

The *lac* operon is found in *E. coli* and some other bacteria. In prokaryotes, an **operon** is a unit of DNA under the regulation of a single promoter that codes for several proteins. The *lac* operon is an example of an **inducible operon** (meaning it can be switched on or off). It expresses three structural genes that code for three enzymes, but only when the sugar **lactose** is available. The enzymes break down lactose into the usable forms glucose and galactose.

Producing the enzymes that break down lactose constitutively would be a misuse of energy for the bacteria, because lactose is not the preferred energy source for *E. coli*. It is important that the *lac* operon genes are inducible and are only expressed when lactose is present in the environment.

The *lac* operon consists of:

- a promoter—the binding site of the RNA polymerase
- an **operator**—the binding site of the transcription factor, which, in this case, is a repressor
- three structural genes—*lacZ* (β-galactosidase), *lacY* (β-galactoside permease) and *lacA* (β-galactoside transacetylase)—that code for three different enzymes (Figure 5.3.4).

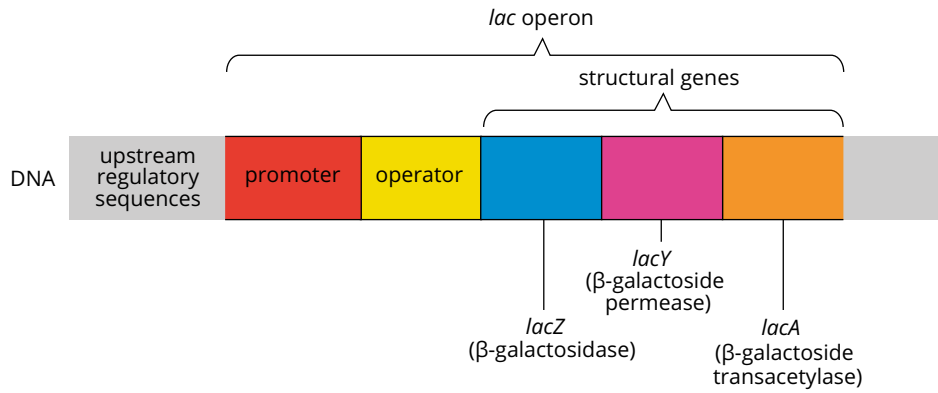


FIGURE 5.3.4 The *lac* operon.

Adjacent to the *lac* operon is the regulatory gene ***lacI***. *LacI* codes for a transcription factor called the ***lac* repressor**. The *lacI* gene is expressed constitutively (or continually), so the *lac* repressor is always present. This transcription factor binds to the operator in the *lac* operon, blocking the RNA polymerase from binding to and transcribing the structural genes in the *lac* operon, preventing the synthesis of the three enzymes involved in lactose metabolism. However, when lactose is present, the lactose binds to the *lac* repressor, inhibiting the transcription factor from binding to the operator. This enables the RNA polymerase to attach to the promoter in the *lac* operon and transcribe the three structural genes, resulting in the production of the enzymes involved in lactose metabolism (Figure 5.3.5).

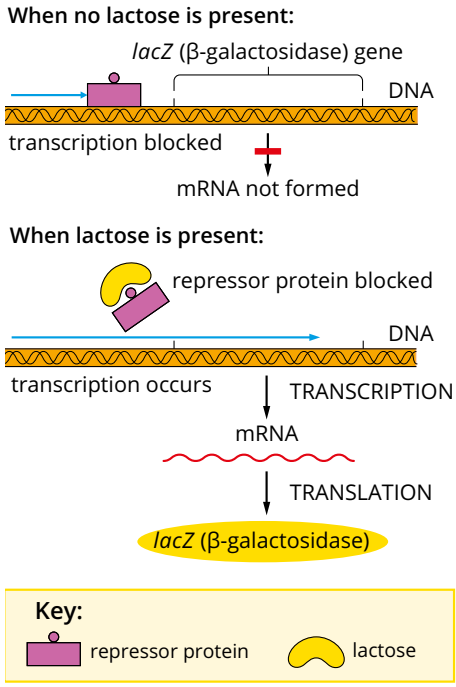


FIGURE 5.3.5 The genes of the *lac* operon are only expressed in the presence of lactose.

Master regulatory genes

The development of a complex, trillion-celled adult organism from a single fertilised cell occurs in a series of steps. Master regulatory genes code for transcription factors that turn genes on and off in different cells in the developing embryo. They can also start a sequence of events by turning other regulatory genes on and off, leading to the production of transcription factors that will, in turn, regulate other genes, and so on. A single master regulatory gene can, in this way, control the development of a complex structure such as an eye or nervous system, or a whole organism.

Some of the most important master regulatory genes are the **homeotic genes**. Homeotic genes control the structure and organisation of body segments during embryonic development. The proteins of homeotic genes bind to regulatory regions of target genes, which then activate or repress cellular activity, directing the development of the organism.

Hox genes

***Hox* genes** are master regulatory genes that affect the spatial pattern of expression of other genes. *Hox* genes control genes that form specific tissues of an organism at specific times during embryonic development. This group of genes determines the body plan along the head-to-tail axis during embryonic development. The expression of these master regulatory genes during the development of an organism determines the structures that will be formed at a given position.

In the fruit fly, *Drosophila*, *Hox* genes determine the position of the abdomen, thorax and head, and the location of the antenna and legs. As an embryo develops, the *Hox* genes direct messages to groups of embryonic cells to, for example, 'grow into a head' or 'develop into an eye'.

A mutation in a single *Hox* gene can lead to a fully functional leg growing where the antenna should be (Figure 5.3.6). Because the production of a leg requires the coordinated action of many genes, the base change must have occurred in a gene that switches the leg genes 'on' and other genes 'off'. This means a *Hox* gene has control over the genes that are involved in the development of all the cell types in both leg and antenna.

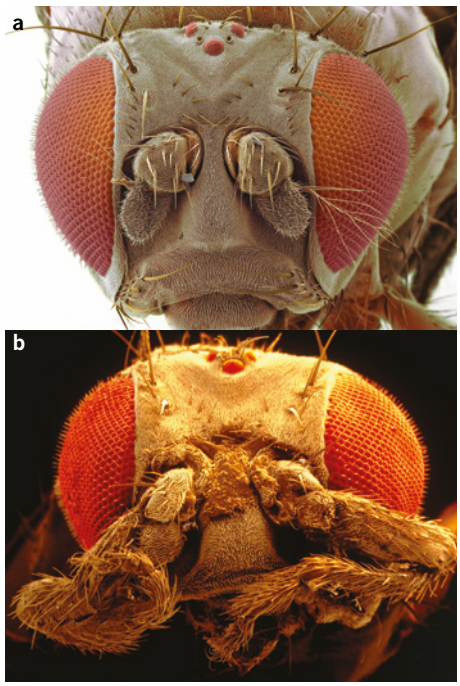


FIGURE 5.3.6 (a) A normal fly. (b) A mutation in a *Hox* gene results in a fly with legs growing where antennae should be.

It is inferred that this important gene family has been highly conserved throughout animal evolution. Because of the high degree of genetic similarity in these genes across animal species, researchers can use model organisms, such as *Drosophila*, to investigate human birth defects and diseases (Figure 5.3.7).

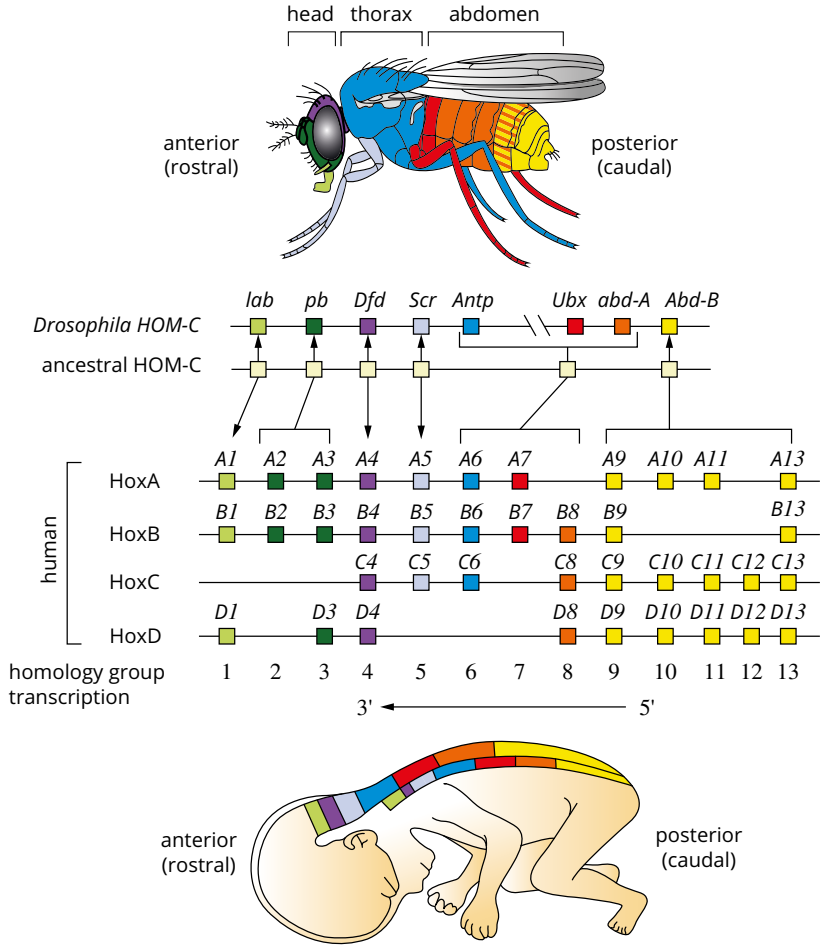


FIGURE 5.3.7 The genomic organisation and expression patterns of the *Hox* gene family in *Drosophila* and humans.

Sex determination

In humans, sex is determined by the presence of a pair of sex chromosomes (Figure 5.3.8). Females have two X chromosomes, and males have one copy of the X chromosome and one copy of the Y chromosome (Figure 5.3.9).

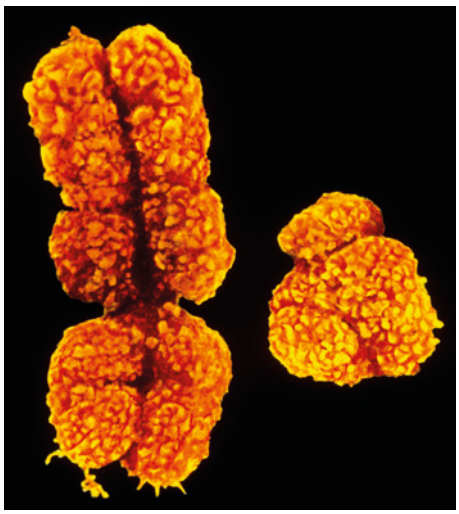


FIGURE 5.3.9 Coloured scanning electron microscope image of human sex chromosomes: X (left) and Y (right).

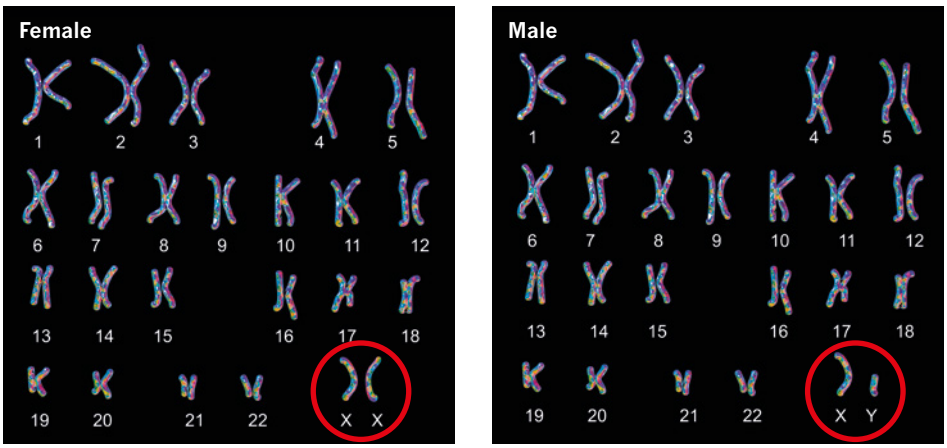


FIGURE 5.3.8 Human karyotype diagrams highlighting the difference between female and males.

One of the major genes for the regulation of sexual characteristics in mammals is found on the Y chromosome. The **sex-determining region Y (SRY)** gene codes for a protein that directs the development of the gonads into testes. Other autosomal genes regulate the embryonic development of the gonads initially.

The *SRY* gene is a 35 000–base pair region on the short arm of the Y chromosome. It codes for a 223–amino acid peptide (Figure 5.3.10) that is thought to be a transcription factor that turns on male genes, but according to current research could also turn off anti-male genes. Recent studies in mice indicate that if the *SRY* gene is not expressed at the correct time, sex differentiation does not occur. There is only a very small period of time for the testes-forming genes to function. If they are not turned on at the correct time during embryonic development, the ovary-forming pathway is activated.

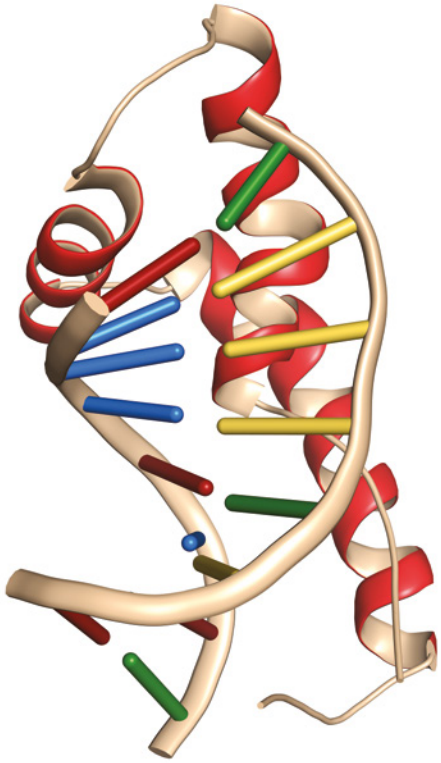


FIGURE 5.3.10 An SRY protein (red, curled) bound to a DNA helix.

Tumour-suppressor genes

Cell differentiation is a complex process controlled by regulatory genes. Information about regulatory genes is constantly developing. Much of what we know about cell differentiation and gene regulation comes from research into cancer.

Cancer is a set of diseases in which cells escape from the control mechanisms that normally limit their growth. Genes that normally regulate cell growth and division during the cell cycle include genes for growth factors, their receptors and molecules for signalling pathways. Mutations that alter any of these genes in cells can lead to cancer.

In addition to genes whose products normally promote cell division, cells contain genes whose normal products inhibit cell division. These genes are called **tumour-suppressor genes** because the proteins they encode for can prevent uncontrolled cell growth.

Tumour-suppressor proteins prevent cancer. These proteins are produced in response to exposure to radiation and chemicals that cause damage to the structure of DNA. Some tumour-suppressor proteins repair damaged DNA, preventing the cell from accumulating cancer-causing mutations.

An important tumour-suppressor protein is p53 (Figure 5.3.11). If DNA damage has occurred, the p53 protein binds to specific sites on the DNA to repress genes that play a role in the continuation of the cell cycle. This inhibits cell division and prevents damaged DNA from replicating. If the damage is minor, p53 activates genes that repair DNA. In cases where the damage cannot be repaired, p53 will initiate cell death (apoptosis).

The p53 protein plays a major role in the prevention of cancers. If the gene coding for the p53 protein is deleted or mutated, the risk of cancer is greatly increased. In 50% of all cancers, p53 has been found to be inactive.

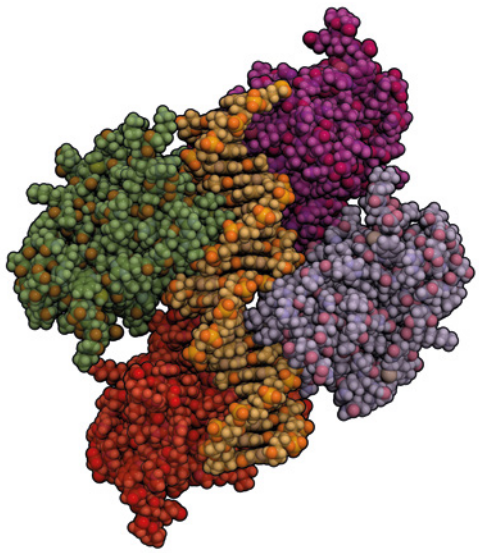


FIGURE 5.3.11 A molecular model of the tumour-suppressor protein p53 (left and right) bound to a DNA molecule (centre)

Alternate splicing

Enzymes in eukaryotic nuclei modify pre-mRNA in specific ways before translation. During this RNA processing (Module 5.2), both ends of the primary transcripts are altered and, in most cases, interior sections of the RNA molecule (introns and exons) are cut out and the remaining parts are spliced together. These modifications produce an mRNA molecule ready for translation.

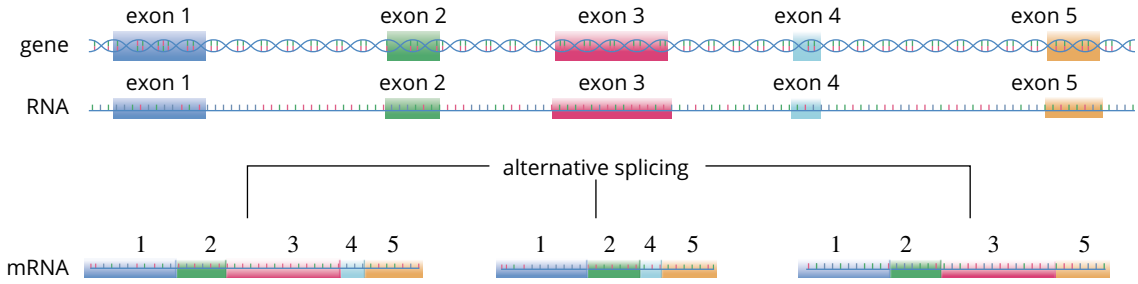


FIGURE 5.3.12 Alternative splicing of a single gene gives rise to alternative mRNA molecules, resulting in many different proteins.

A pre-mRNA transcript can be spliced in many different ways, resulting in alternative mature mRNA strands from a single gene and therefore different proteins. This is the result of some exons being removed along with the introns during RNA processing. For example, a particular gene may result in a mature mRNA that contains all exons 1–5, but the same gene may result in another mature mRNA that contains only exons 1, 2, 4 and 5 (Figure 5.3.12). Alternative splicing is one reason that the 21 000 genes of humans are able to produce many more than 21 000 proteins.

In early research on gene structure, introns were called 'junk DNA' because it was believed they had no role in protein production. It is now known that protein expression is much more complex than first thought and that, by splicing mRNA during RNA processing, different proteins may be made from the same gene.

Epigenetic inheritance

The structure of the DNA was described in Module 5.1. The DNA of eukaryotic cells is packaged with proteins, called **histones**, in an elaborate complex known as **chromatin**. The structural organisation of chromatin not only packs a cell's DNA into a compact form that fits inside the nucleus, but also helps regulate gene expression.

Histones are proteins found in eukaryotic cells that tightly package DNA into structures called nucleosomes.

Epigenetics can be defined as any mechanism that alters gene expression without altering the DNA sequence. The changes to the chromatin structure can be passed onto future generations through the maternal lineage, but the DNA sequence is not altered. Inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called **epigenetic inheritance**.

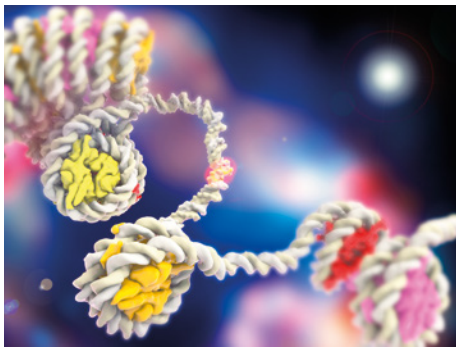


FIGURE 5.3.13 DNA strands coil around histones to form nucleosomes.

The DNA strands coil around histones to form circular structures called **nucleosomes** (Figure 5.3.13). Epigenetic modifications include the addition of methyl or phosphate groups to the DNA or histones in the chromatin. These chemical changes affect how DNA is coiled and result in changes in gene expression and variations in phenotype because of changes in the transcription and translation processes. These changes are a result of environmental factors.

It is now understood that living processes, both intracellular and extracellular, involve control by central information (the genome) and the ability to adapt within the limits of cellular machinery and chemistry. This is seen in phenotypic changes, such as the upregulation of melanin to darken the skin in response to sunlight stress.

Epigenetic modifications do not change the genetic code, which is bound by the central information, but they do allow the expression of genes to be flexible. The study of epigenetics and molecular markers that alter the expression of genes can be used to potentially identify biological mechanisms that cause diseases.

Twin studies

Monozygotic (identical) twin studies contribute to our understanding of the genetic component of age-related traits and diseases. Because identical twins have developed from the same fertilised egg, they have the same genome. Twins who are raised together also have shared environments, therefore any difference in their phenotype must be due to epigenetic modification. Epigenetic variations might explain, for example, why one identical twin acquires a genetically based disease such as schizophrenia, but the other does not, despite them having identical genomes.

As twins age, they acquire different characteristics as a result of their environment. It has been proposed that these phenotypic differences are the result of epigenetic differences accumulating in the chromatin of the twins. The older the twins become, the greater the number of differences in the histones surrounding the DNA that regulates gene expression. Research indicates that not all age-related diseases are heritable. For every heritable disease, there is also an important non-genetic component that may influence the onset and severity of the disease.

Different responses to diseases in identical twins may be due to differences in their embryonic development or the ways their bodies respond to environmental factors. These responses could be random somatic mutations at any of the stages of DNA replication or protein synthesis processes. The use of identical twins allows researchers to control for contributing factors such as age, gender, maternal effects and most in utero and environmental influences.

While there is a significant body of evidence available to suggest that the less time twins spend together, the greater the epigenetic variability, further research is required to determine how environmental changes directly cause the epigenetic change in the chromatin. Smoking is one factor that has been reliably detected as a significant factor in changing the epigenetic pattern.

New technology, such as CRISPR (discussed in Chapter 8), combined with an improved understanding of the epigenetic markers that contribute to disease, can potentially allow for targeted epigenome editing and reprogramming of genes. In the near future it may be possible to manufacture more effective therapeutic drug treatments for cancer based upon our understanding of epigenetics. We may be able to repair or replace mutations in genes, such as the tumour-suppressor gene and other oncogenes.

SAT IA3

5.3 Review

SUMMARY

- Gene expression in eukaryotes can be regulated at any of the three stages: transcription, RNA processing and translation.
- Gene regulation in prokaryotes occurs during transcription.
- Constitutive genes are expressed continually.
- Transcription of other genes can be induced or repressed as needed by transcription factors, which is a form of gene regulation.
- Regulatory genes code for the production of transcription factors. Transcription factors are proteins that control gene expression at the transcription stage.
- Structural genes code for proteins and RNAs that are not involved in gene regulation.
- Cell differentiation and regulation of cell processes is a complex process requiring regulatory genes, and homeotic genes.
  - The *lac* operon in *E. coli* is an example of a unit of DNA for which transcription can be regulated.
  - Master regulatory genes code for transcription factors that turn genes on or off during embryonic development.
  - The *Hox* gene is an example of a master regulatory gene that determines the spatial arrangement of body parts to determine the body plan of an organism.
  - The *SRY* gene, which helps determine sex in mammals, is located on the Y chromosome. It must be expressed at the appropriate time for the testes-forming genes to function.
  - Tumour-suppressor genes inhibit cell division, and so prevent uncontrolled cell growth that leads to cancer.
  - Alternative splicing is one method where eukaryotic cells can modify pre-mRNA and increase the complexity and variety of proteins coded for by the same gene.
  - Epigenetic variation alters gene expression without altering the DNA sequence. Chemical changes to the histones surrounding the nucleosomes affect gene expression in response to changes in environmental factors.
- There are many differences in the structure of the genetic information of prokaryotes and eukaryotes. These structural differences affect the way in which genetic information is transcribed, translated and expressed.

KEY QUESTIONS

Retrieval

1 Describe the structure of a nucleosome.

2 Define the following terms:
- **a** transcription factors
- **b** regulatory gene
- **c** constitutive gene
- **d** induced gene

3 Identify the name given to continually functioning genes within cells.

4 Describe the function of the following transcription factor genes:
- **a** *Hox* genes
- **b** SRY genes

Comprehension

5 Explain the importance of regulatory genes for cell differentiation, tissue formation and morphology.

6 Identify some of the main differences in gene regulation between prokaryotic and eukaryotic cells.

7 Explain why some genes are continually 'on', while others are not.

8 Complete the table by inserting in the correct order the processes that occur in *E. coli* in the presence and absence of lactose.

Presence of lactose in the environment	Absence of lactose in the environment

- inhibition of gene expression
- production of the repressor
- intake of lactose into the cell
- repressor binds to the operator
- inhibition of repressor activity
- breakdown of lactose
- gene expression

Analysis

9 Tryptophan is an amino acid that prokaryotic cells are able to synthesise when it is in short supply in the environment. Tryptophan production is controlled by a series of five enzymes that are coded for by five genes (*Trp A*, *B*, *C*, *D* and *E*), which form a single unit called the tryptophan operon. This operon has been extensively studied in the bacterium *E. coli*. The *trp* operon, and some of its upstream region, is shown below.

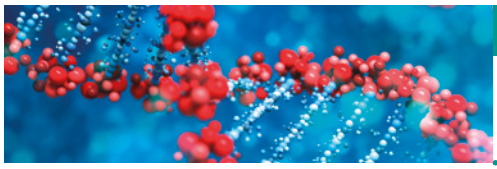
The gene for the repressor protein is constitutively expressed and is produced in an inactive form. It becomes activated when it binds to tryptophan.

a Deduce the expression of genes when *E. coli* has an abundance of tryptophan in its diet.

b Compare the means of repression of the *lac* operon and the *trp* operon.

10 Single-celled organisms such as amoeba often live in environments that change quickly. Consider how the control of gene expression ensures that an amoeba is able to respond effectively to frequent short-term environmental change.

5.4 Mutations in genes



BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- identify how mutations in genes can result from errors in DNA replication, cell division and damage by mutagens.

Changes to the genetic information of a cell is called a mutation. These changes can be due to the rearrangement of long sections of DNA or alterations in one or a few nucleotide pairs.

Mutations result in different forms of the same gene. These different forms are known as alleles. When different alleles of the same gene are examined using DNA sequence analysis, differences in the DNA base pair sequence are detected. Some alleles have changes in the DNA sequence that do not affect the structure of the polypeptide product of the gene but only affect the rate of gene expression. At the other extreme, some mutations create alleles that produce non-functional polypeptides.

In this module, you will learn about changes due to transcriptional errors and block mutations and how these mutations can have an effect on the individuals carrying them.

MUTATIONS

A **mutation** is a change in the sequence of nucleotides in DNA. Mutations can occur in small parts of the DNA sequence. They can also involve duplication or deletion of a whole or part of a chromosome or changes in chromosome structure. It is important to remember that mutation means change.

Mutations can occur randomly during replication. Mutations may affect a single gene, multiple genes or entire chromosomes. They can occur spontaneously or as a result of **mutagens**—factors that induce mutation. Common mutagens include different forms of radiation such as ultraviolet (UV) radiation.

Most mutations are detected and repaired by enzymes. Those that cannot be repaired fall into one of the three categories: neutral, harmful or beneficial.

- Neutral mutations have no effect on survival.
- Harmful mutations decrease the likelihood of survival.
- Beneficial mutations increase the likelihood of survival.

Once formed, a mutation is established in the DNA unless changed by another mutation. The study of mutations is a growing field in biology (genetics) and bioinformatics. Current research demonstrates that cytosine-to-thymine mutations occur more often than any other, and that mutations are more likely to occur in clusters within a gene or across several related genes.

One mutation database has over 645 000 harmful human mutations identified from research. It also has over 5 227 000 DNA regions with approximately 34 870 900 human mutations, many of which are of unknown phenotypic function. These are classified as neutral mutations until they are further understood

Beneficial mutations are dependent on population genetics and natural selection (Chapter 9). They are currently modelled, as there is no human database available (although many are being investigated). It is hypothesised that a mutated part of DNA is no more likely to change than any other part of the DNA.

Somatic mutations occur in body cells. They only affect the individuals in which they arise and cannot be inherited by future generations. **Germline mutations** are heritable because they affect gametes (meaning they occur in the DNA of ova or sperm), and can be passed on to offspring. A germline mutation may bring a new allele into a gene pool, potentially influencing the allele frequencies in a population.

Types of mutations

The alteration of a single base in DNA is referred to as a **point mutation** (Figure 5.4.1) and every amino acid change results from a change in the base sequence of DNA (Figure 5.4.2).

FIGURE 5.4.1 A point mutation involves just one nucleotide in RNA (or one nucleotide pair in DNA).

a Normal sequence

DNA sequence transcribed	TAC	ATA	CAT	ATA	CAT	ATA	CAT
mRNA sequence	AUG	UAU	GUA	UAU	GUA	UAU	GUA
Amino acid sequence	Met	Tyr	Val	Tyr	Val	Tyr	Val

b Synonymous mutation (base substitution → no amino acid replacement)

Normal DNA sequence	TAC	ATA	CAT	ATA	CAT	ATA	CAT
Mutant DNA sequence	TAC	AT**G**	CAT	ATA	CAT	ATA	CAT
mRNA sequence	AUG	UA**C**	GUA	UAU	GUA	UAU	GUA
Amino acid sequence	Met	**Tyr**	Val	Tyr	Val	Tyr	Val

c Non-synonymous substitution mutation (base substitution → amino acid replacement)

Normal DNA sequence	TAC	ATA	CAT	ATA	CAT	ATA	CAT
Mutant DNA sequence	TAC	**G**TA	CAT	ATA	CAT	ATA	CAT
mRNA sequence	AUG	**C**AU	GUA	UAU	GUA	UAU	GUA
Amino acid sequence	Met	**His**	Val	Tyr	Val	Tyr	Val

d Nonsense mutation (base substitution → new 'stop' codon shortens the polypeptide)

Normal DNA sequence	TAC	ATA	CAT	ATA	CAT	ATA	CAT
Mutant DNA sequence	TAC	ATA	CAT	AT**T**	CAT	ATA	CAT
mRNA sequence	AUG	UAU	GUA	UA**A**	GUA	UAU	GUA
Amino acid sequence	Met	Tyr	Val	**STOP**			

e Single base insertion (frameshift—changes all amino acids after the point of insertion)

Normal DNA sequence	TAC	ATA	CAT	ATA	CAT	ATA	CAT	
Mutant DNA sequence	TAC	ATA	**G**CA	TAT	ACA	TAT	ACA	T
mRNA sequence	AUG	UAU	**C**GU	AUA	UGU	AUA	UGU	A
Amino acid sequence	Met	Tyr	**Arg**	**Ile**	**Cys**	**Ile**	**Cys**	

f Single base deletion (frameshift—changes all amino acids after the point of deletion)

Normal DNA sequence	TAC	ATA	**C**AT	ATA	CAT	ATA	CAT
Mutant DNA sequence	TAC	ATA	_ATA	TAC	ATA	TAC	AT
mRNA sequence	AUG	UAU	UAU	AUG	UAU	AUG	UA
Amino acid sequence	Met	Tyr	**Tyr**	**Met**	**Tyr**	**Met**	

FIGURE 5.4.2 A portion of the DNA template strand of a hypothetical gene showing the effect of different types of point mutations on the amino acid chain.

However, not every change in the DNA base sequence results in an amino acid change. For example, the DNA sequence ATA is transcribed to the mRNA sequence as UAU and specifies the amino acid tyrosine. A mutation producing the DNA sequence ATG results in the mRNA sequence UAC, which still specifies tyrosine (Figure 5.2.8 on page 266). Mutations like this, which have no effect on the polypeptide, are called **synonymous mutations** (Figure 5.4.2b). The DNA strand will have an optimal sequence of nucleotides to produce functional proteins. When there is a change in the DNA sequence that results in a different codon, but codes for the same amino acid, the structure of the mRNA will differ.

This post-transcriptional difference between the optimal codon sequence and the mutation codon is thought to affect the stability of the mRNA and the way that it is spliced during processing. There is now sufficient experimental evidence to suggest that these changes have a significant effect on the efficiency of gene expression and protein synthesis, and may be responsible for triggering disease and cancer.

Synonymous mutations do not result in an amino acid change. However, they do affect the messenger RNA structure and therefore may change the rate of protein expression, conformation and function.

Sickle-cell anaemia

Point mutations that result in an amino acid replacement are called **non-synonymous substitution mutations** (Figure 5.4.2c on page 287). Most people have normal haemoglobin in their red blood cells. However, some people have a type of haemoglobin that precipitates when oxygen concentration in the blood falls, resulting in distorted 'sickle-shaped' red blood cells (Figure 5.4.3). The genetic basis for sickle-cell anaemia is a mutation of a single nucleotide pair in the gene that encodes the beta-(β)-globin polypeptide of haemoglobin.

Haemoglobin consists of four polypeptide chains: two alpha (α) and two β-chains produced from α-globin and β-globin genes respectively. The difference between normal and sickle-cell haemoglobin is a single amino acid alteration in the β-chain, which changes the shape of the molecule. This chain consists of 146 amino acids. If the amino acid sequences of the polypeptide of β-chains of normal individuals and those with sickle-cell anaemia are compared, only one difference in amino acid sequence is observed.

The replacement of glutamic acid (in normal haemoglobin) with valine (in sickle-cell haemoglobin) dramatically alters the structure of the haemoglobin protein, changing the shape of red blood cells (Figure 5.4.3). This has serious consequences because it reduces the capacity of the haemoglobin molecule to carry oxygen to tissues. All the other amino acids in the polypeptides are identical.

This single point mutation has a major effect on those people with the sickle-cell gene. People with sickle-cell anaemia will die if not treated quickly when they show symptoms of the disease. Even with treatment, they seldom live beyond puberty. People who suffer from sickle-cell anaemia or who have the sickle-cell trait are very rare in Australia but its prevalence has been increasing due to immigration of people from other countries where the genetic disorder is more common.

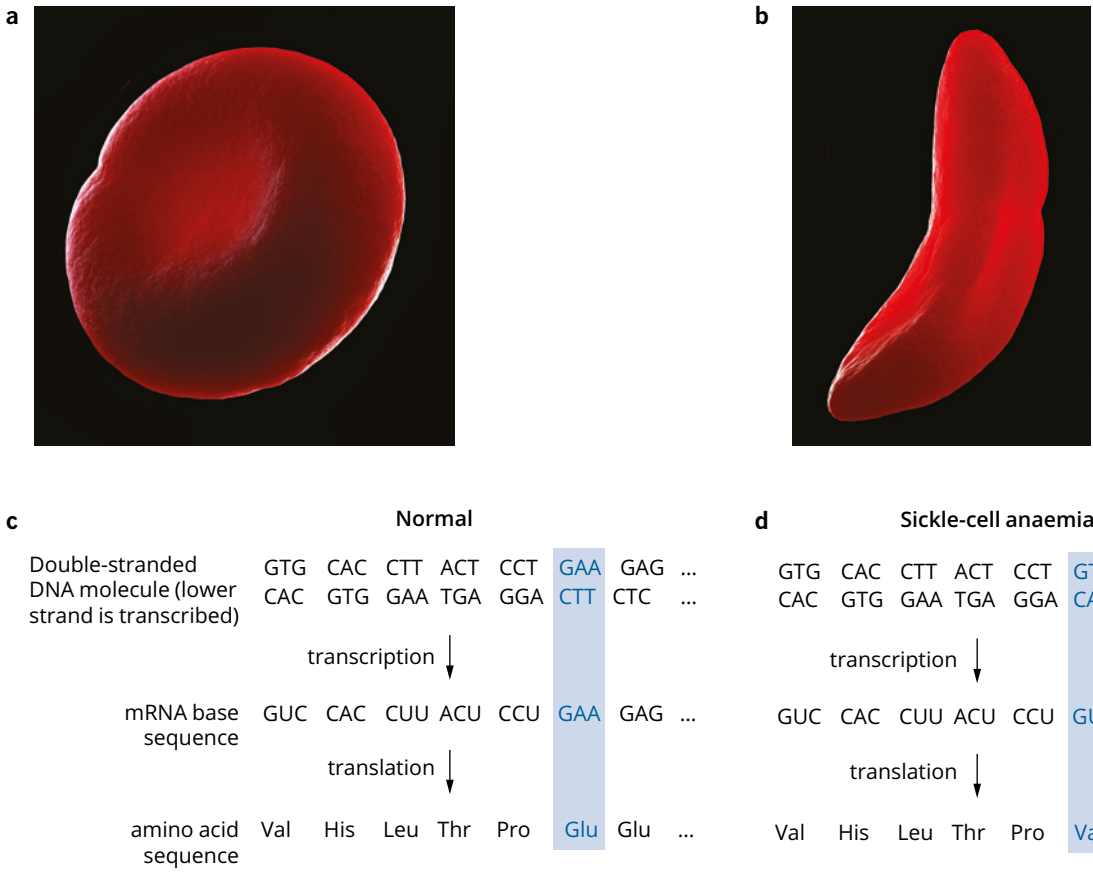


FIGURE 5.4.3 Red blood cells of (a) normal individuals and (b) those with sickle-cell anaemia. DNA, messenger RNA and the resulting amino acid sequences of the β-chain of haemoglobin proteins of (c) normal individuals and (d) those with sickle-cell anaemia.

Mutations that result in the generation of a 'stop' codon—for example UAU (tyrosine) to UAA (stop)—are termed **nonsense mutations** because no further amino acids are added after the site of mutation (Figure 5.4.2d on page 287). Because nonsense mutations stop translation, they may have severe effects, particularly if the mutation occurs early in a gene. Nearly all nonsense mutations lead to non-functional proteins.

If a single base pair is added to or deleted from the DNA, the reading frame (codon sequence) of the mRNA is altered, and the wrong amino acids will be incorporated for the remainder of the sequence (Figure 5.4.2.e, f on page 287). This type of change is called a **frameshift mutation** if the number of nucleotides inserted or deleted is not a multiple of three. In Figure 5.4.2e (page 287), the base guanine (G) is added to the sixth position from the left. In Figure 5.4.2f (page 287), the base cytosine (C) is deleted from the sixth position from the left.

Frameshift mutations can have very significant effects on the polypeptide formed. All amino acids after the point of a frameshift mutation will be improperly grouped into codons, and the result will be extensive missense, usually ending sooner or later in nonsense and premature termination. Unless the frameshift is very near the end of the gene, the protein is almost certain to be non-functional.

WS 4.1.1

Cystic fibrosis

The addition or deletion of bases from DNA results in mutations that can have very significant effects. In the disease **cystic fibrosis**, an affected individual carries two mutant alleles of the *CF* gene on chromosome 7. The normal copy of the *CF* gene produces a protein named CFTR (an abbreviation of cystic fibrosis conductance regulator), which forms a channel in the outer cell membrane. The CFTR channel controls the flow of chloride out of the cell. Mutations in the gene can lead to defective CFTR channel proteins being produced or, for extreme alleles, no protein being produced.

Cystic fibrosis symptoms vary from person to person. Affected individuals have high salt levels in their sweat and frequently produce sticky mucus in the lungs, which blocks the airways and increases the chance of infection. The pancreas does not work efficiently in most sufferers and some individuals may also have liver problems.

The CF polypeptide consists of 1480 amino acids. Molecular analysis of the DNA of individuals with cystic fibrosis has shown that one particular mutant allele, called Δ*F508*, is common. It is present in up to 90% of cases. The Δ*F508* allele has a deletion of three base pairs found in the normal copy of the *CF* gene (Figure 5.4.4). The deletion of the three base pairs results in the deletion of the amino acid phenylalanine from the polypeptide produced by the Δ*F508* allele.

The loss of one amino acid out of 1480 may not seem significant, but it is. In fact, it is so significant that the cell recognises the polypeptide produced by the Δ*F508* allele as being defective and it is destroyed before it gets to the cell membrane.

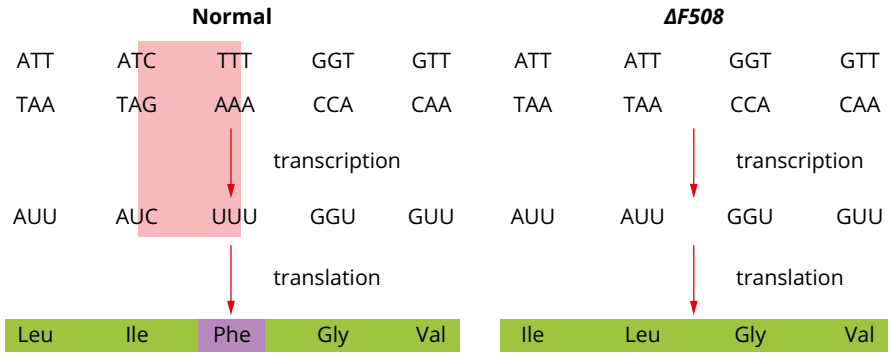


FIGURE 5.4.4 Comparison of the base messenger RNA and DNA sequences and the resulting amino acid sequences of the CFTR protein from normal individuals and those with cystic fibrosis.

A beneficial mutation

A possible example of a beneficial substitution point mutation is one that involves the *APOA-1* gene. This gene codes for a protein (apolipoprotein A-1) that is normally involved in the transport of cholesterol and phospholipids to the liver, where they are then redistributed or broken down and excreted. One of the mutated forms of the protein, ApoA-1 Milano, involves a substitution of the amino acid arginine for cysteine. This mutated protein acts as an antioxidant, reducing cholesterol deposition in arteries and significantly decreasing the risk of cardiovascular disease.

More research is being done on apolipoprotein A-1 mutations to understand its correlation with familial visceral amyloidosis (an abnormal accumulation of proteins, amyloidosis, in internal organs). The liver, kidneys and heart are commonly affected by amyloidosis.

The mutant form of the ApoA-1 protein (Figure 5.4.5) was first identified in Milan, which is why the mutated gene was named after that city. Further investigation, including blood tests of an entire Italian village, traced the origin of the germline mutation to a single man. The 3.5% frequency of the gene in that village population can be attributed to the descendants of this one man.

To be considered a beneficial mutation, natural selection will have to result in an increase in the frequency of this allele in the population, to the advantage of the species (Chapter 9).

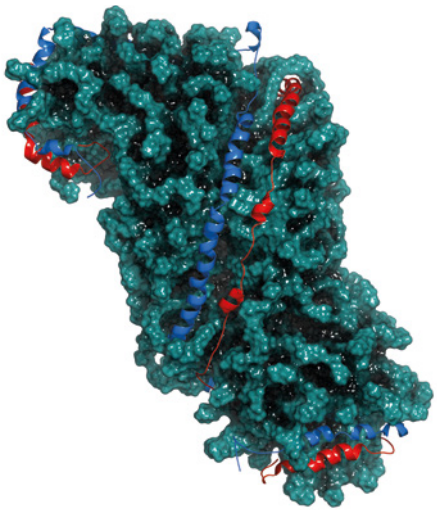


FIGURE 5.4.5 ApoA-1 Milano is a mutated form of a protein that can reduce cholesterol levels in the human blood stream. ApoA-1 Milano is caused by a beneficial point mutation.

Causes of mutation

We have considered several types of mutation that can occur in DNA. There are a number of different ways in which these can arise.

Spontaneous mutations

Spontaneous mutations arise naturally as random changes in the base sequence of DNA. They may occur as a result of rare, undetected and unrepaired errors of DNA synthesis. Spontaneous mutations occur at an average rate of approximately one in a million; that is, an error occurs in a given gene about once in every million base pairs replicated.

Induced mutations

Mutations result from random changes to DNA and the agents that induce mutations are called mutagens. The rate of mutation can be increased above spontaneous (background) levels by exposing cells to biological, chemical or physical mutagens (Figure 5.4.6). Mutations that occur following deliberate or accidental exposure to mutagens are called **induced mutations**.

Induced mutations can occur more frequently than spontaneous mutations. During evolutionary history, it is thought that organisms have been exposed to many naturally occurring mutagenic chemicals and sources of radiation in the environment. These background effects have contributed to the spontaneous mutation rate.

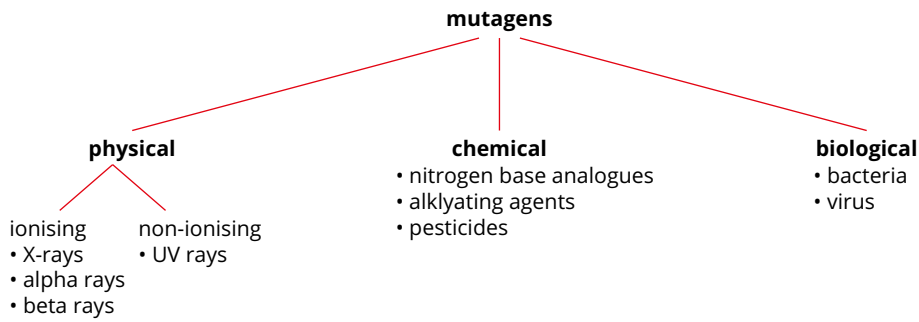


FIGURE 5.4.6 Types of mutagens.

Biological mutagens, such as viruses and bacteria, change the genetic composition of the cells by integrating their DNA into the human genome during cell division. This can lead to changes in the normal functioning of the genes.

Chemical mutagens can interfere with correct DNA replication by inserting themselves into the DNA and distorting the double helix. Chemicals that are chemically similar to the nitrogen bases can substitute into the DNA strand and result in faulty base pairing, misreading of the DNA sequence and disruption of the replication machinery. Other chemical mutagens may modify the nitrogen bases by deamination or alkylation processes. These processes significantly disrupt the regular base pairing and introduce frameshift mutations. Some pesticides act as chemical mutagens, and the resulting base pair changes have been linked to neurodegenerative diseases such as Parkinson's disease.

Since the industrial revolution, and more dramatically in recent decades, the number of chemicals added to the environment as a result of agricultural and industrial practices and warfare has increased. Some of these chemicals are mutagens, some are **carcinogens** (cause cancer) and some are both. Carcinogens cause an increase in cell division and uncontrolled growth of the affected tissue. This does not always involve direct changes to the genetic material. If it does, then the inducing agent is also a mutagen. Mutations in some genes called **oncogenes** can cause cancer. A mutagen affecting these genes is, therefore, also a carcinogen.

Radiation as a physical mutagen

Physical mutagens include forms of ionising and non-ionising radiation. The particles released as a result of radiation emit sufficient energy to disrupt the atoms or molecules in the DNA structure.

Forms of radiation that are encountered regularly are UV radiation (via sunlight), X-rays (used in medical diagnosis) and other forms of high-energy radiation. Normally, the mutagenic effects of radiation are relatively minor. However, overexposing your body to UV radiation—for example, by sunbathing at the beach—can result in mutagenesis and skin cancer.

Public awareness of the risk of overexposure to UV radiation has increased dramatically in Australia in recent times. The Sun Smart campaign has had a significant influence. UV radiation may damage DNA, causing bases of the same strand to join together, or it may cause the loss of a base. These changes cause distortions to the DNA strand and when DNA replication takes place, the distortions interrupt it, leaving a gap in the strand being synthesised. This results in a mutation in the somatic cells causing cancers like melanoma (Figure 5.4.7).

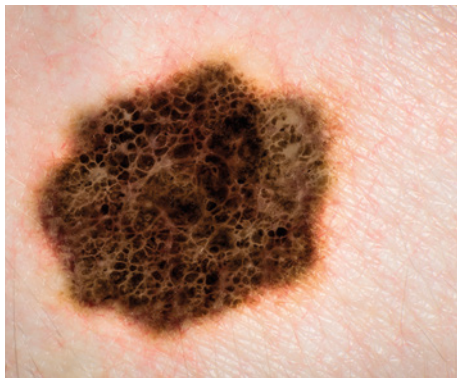


FIGURE 5.4.7 Malignant melanoma skin cancer.

Radiologists wear lead-lined protective clothing to reduce their long-term exposure to X-rays when treating patients. The protective equipment covers the genital regions, to reduce the chance of causing mutation to the DNA in the germline cells (eggs or sperm). X-rays, which have a higher energy than UV radiation, can break DNA and even cause chromosome breakage, resulting in cell death or mutational change. Radiation from radioactive elements can have similar effects. Both spontaneous and induced mutations may result in similar genetic changes.

Heat as a mutagen

The DNA molecule is a long-chain polymer of nucleotides linked by phosphodiester bonds between the sugar molecule and the phosphate group (Module 5.1). The chemical bond that links the nitrogen base to the sugar molecule is called a β-glycosidic bond. When DNA is exposed to heat, this bond between the deoxyribose sugar and the base can be broken. The nitrogen base is removed from the nucleotide leaving a 'baseless' site in the DNA strand. The SP (sugar–phosphate only) site is unstable and rapidly degrades leaving a gap in the DNA template.

It is thought that as many as 10 000 SP sites are produced in each human cell each day. Despite this, heat mutagenesis is not a common form of mutation because the cell has very effective repair mechanisms in place. However, heat mutagenesis may be responsible for the decline in male fertility, as a result of testes being exposed to increased temperatures, affecting spermatogenesis and the production of viable sperm.

DNA repair

All cells have DNA repair systems, which are important to cell survival and frequently called into action. Cells have enzymes that can repair radiation damage to DNA. If damage to the template strand of a DNA molecule is not repaired, the cell dies. In most cases, DNA repair occurs during replication by randomly incorporating bases into the gap (Figure 5.4.8). If the base incorporated is not the same as the missing base, this results in a mutation in the synthesised strand.

There are also backup repair systems that are used in emergencies when normal repair mechanisms fail. For example, bacteria have an SOS repair system, which acts as an effective last resort when there has been large-scale damage to DNA.

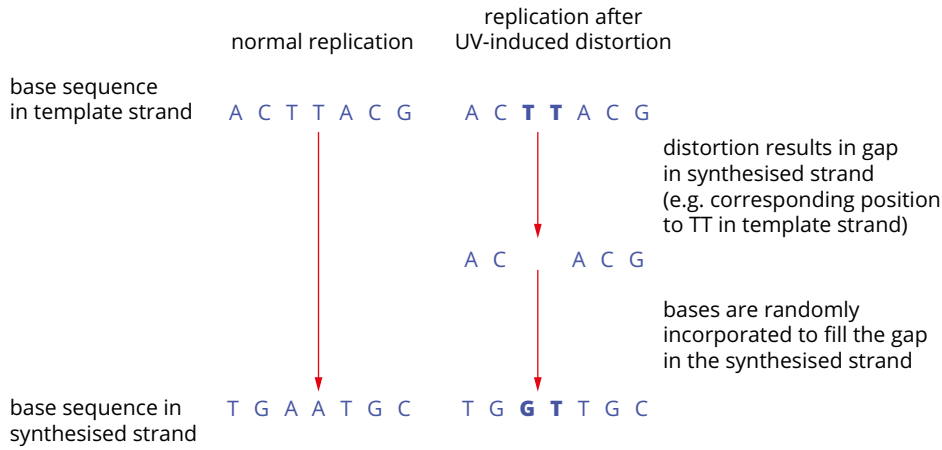


FIGURE 5.4.8 UV damage to DNA may result in bases of the same strand being joined together. This causes a gap in the DNA strand that is filled in by randomly incorporated bases. In this case, a mutation occurs in the synthesised strand. The base sequence during normal replication is shown for comparison.

PA 4.1.2

5.4 Review

SUMMARY

- A change in the genetic information of a cell is called a mutation.
- Mutations result from a change in the sequence of nucleotides in the DNA.
- Most mutations are repaired by enzymes, so not all mutations are harmful. Some mutations will have no effect on the cell processes and others may benefit the organism.
- Mutations can be classified as:
  - point mutations (a single change in a nucleotide in the DNA, such as a deletion, substitution or insertion)
  - synonymous mutations (produce no effect on the peptide chain).
- Some genetic diseases result from mutations.
  - Sickle-cell anaemia is the result of a substitution point mutation that disrupts the structure of the haemoglobin molecule and prevents the effective oxygen-carrying capacity of the affected individual's red blood cells.
  - The most common form of cystic fibrosis is the result of the deletion of three base pairs in the *CF* gene, which causes a defect in the chloride channels in the cell membranes of the affected individual.
- Mutations can be spontaneous, random changes occurring during replication or induced by mutagens in the environment. Mutagens can be physical (e.g. radiation), chemical (e.g. pesticides) or biological (e.g. bacteria and viruses).

KEY QUESTIONS

Retrieval

1 Define mutation.

2 Recall the name of mutations that are heritable.

Comprehension

3 Explain why single nucleotide substitution mutations are usually much less damaging than frameshift mutations in functional encoding genes.

Analysis

Use the strand of DNA shown here and the codon chart in Figure 5.2.8 (page 266) to answer the next question.

3' TAC GCA AGC AAT ACC GAC GAA 5'

Position 1 refers to the first base at the 3' end of the transcribed DNA strand. The last base in the DNA strand, at the 5' end, is at position 21.

4 **a** The following table lists five point mutations that may occur in the original strand of DNA. Determine the effects on both the DNA and amino acid sequence produced as a result of each mutation. Justify each response with reference to both the sequence change and the amino acid sequence changes.

Five point mutations that may occur in sample strand of DNA

Mutation	Effect on DNA and amino acid sequence
substitution of T for G at position 8	
addition of T between positions 8 and 9	
deletion of C at position 15	
substitution of T for C at position 18	
deletion of C at position 18	

b Conclude which of the mutations produces the greatest change in the amino acid sequence of the polypeptide coded for by this 21 base pair gene.

5 Compare the effect of the following mutagens on DNA structure and function: UV radiation, ionising radiation, heat, chemical and biological mutagens.

Chapter review

05

KEY TERMS

5' cap
adenine (A)
allele
anticodon
antiparallel
base
carcinogen
chromatin
chromosome
coding DNA
coding strand
codon
complementary base pairing
condensation polymerisation
constitutive gene
cystic fibrosis
cytosine (C)
degenerate
deoxyribonucleic acid (DNA)
deoxyribose
diploid
double helix
epigenetic inheritance
epigenetics
exon
frameshift mutation
gene
gene expression
gene regulation
genetic code
genome
germline mutation
guanine (G)
haemoglobin
haploid
heterogametic
histone
homeotic gene
homogametic
Hox gene
induce
induced mutation
inducible operon
intron
lac operon
lac repressor
lacI
lactose
locus
messenger RNA (mRNA)
monomer
mutagen
mutation
nitrogenous base
non-synonymous substitution mutation
noncoding DNA
nonsense mutation
nucleosome
nucleotide
oncogene
operator
operon
plasmid
ploidy
point mutation
poly-A tail
promoter region
protein
protein synthesis
purine
pyrimidine
regulatory gene
repressed
ribonucleic acid (RNA)
ribose
ribosomal RNA (rRNA)
RNA polymerase
RNA processing
sex-determining region Y (SRY)
somatic mutation
spliceosome
splicing
spontaneous mutation
structural gene
synonymous mutation
TATA box
template strand
thymine (T)
trait
transcription
transcription factor
transfer RNA (tRNA)
translation
triplet
tumour-suppressor gene
uracil (U)

KEY QUESTIONS

Retrieval

1 The three parts of a nucleotide are:
- **A** sugar, phosphate, base
- **B** phosphate, base, protein
- **C** thymine, sugar, protein
- **D** protein, sugar, phosphate

2 Identify the correct statement regarding the structure of DNA.
- **A** Deoxyribose is a six-carbon sugar.
- **B** The base C always pairs with the base G.
- **C** The building blocks are called nucleosomes.
- **D** The DNA molecule is a single-stranded helix.

3 In polypeptide synthesis, the function of the ribosome is to:
- **A** synthesise amino acids
- **B** ensure that DNA transcription is complete
- **C** provide the energy needed for the synthesis
- **D** enable the information in the mRNA to be translated into a chain of amino acids

4 Identify which of the following statements is true about the DNA code for the synthesis of polypeptides:
- **A** The code is read as sets of three bases called triplets.
- **B** Every codon codes for its own exclusive amino acid.
- **C** Each triplet codes for at least two different amino acids.
- **D** There are 20 different amino acids, therefore there are 20 different codons.

5 Identify the name given to the nucleotide sequences that are included in the final mRNA product.
- **A** exons
- **B** introns
- **C** termons
- **D** spliced codons

6 A promoter is:
- **A** a specific sequence of DNA to which DNA polymerase may bind
- **B** a specific sequence of DNA to which RNA polymerase may bind
- **C** a specific sequence of DNA to which a repressor may bind
- **D** a specific sequence of RNA in mRNA

7 In the nucleosome, the DNA is wrapped around:
- **A** histones
- **B** ribosomes
- **C** satellite DNA
- **D** polymerase molecules

8 Describe the difference between the DNA of eukaryotic cells and prokaryotic cells.

9 Describe the function of the codons UAA and AUG.

10 Explain the effect of a single base pair deletion in the DNA of a gene.

11 Sketch a labelled diagram to symbolise a:
- **a** nucleotide with adenine as its base
- **b** strand of DNA, showing the base pairing

Comprehension

12 Identify the three main types of RNA involved in transcription and translation. Summarise the differences between the types of RNA by completing the table.

Type of RNA	Where it is produced	Where and how it functions in cells

13 Identify the correct order of these cell features from largest in size to smallest.
- **A** nucleus, cell, chromosome, gene, base, nucleotide
- **B** cell, nucleus, chromosome, nucleotide, base, gene
- **C** nucleus, chromosome, cell, nucleotide, base, gene
- **D** cell, nucleus, chromosome, gene, nucleotide, base

14 Identify the genetic structure represented by the nucleotide sequence A G U G A C C A A.
- **A** a sequence of mRNA
- **B** a section of double helix
- **C** the amino acid chain of a polypeptide
- **D** part of the DNA template of a particular gene

15 In analysing the number of different bases in a DNA sample, determine which formula would be consistent with the base-pairing rules.
- **A** A = G
- **B** A = C
- **C** G = T
- **D** A + T = G + T
- **E** A + G = T + C

16 Geneticists used DNA sequencing to discover the base sequence of a plasmid. If 27% of the bases in the plasmid were cytosine, determine the correct ratio of base pairs in the plasmid.
- **A** 27% cytosine–guanine pairs
- **B** 46% cytosine–guanine pairs
- **C** 23% adenine–thymine pairs
- **D** 46% adenine–thymine pairs

17 The template strand of a gene contains this sequence:

3'-T T C A G T C G T-5'

Sketch the noncoding DNA sequence and the mRNA sequence, indicating the 5' and 3' ends, then compare the two sequences.

18 The template strand of a gene includes this sequence:

3'-T A C T T G T C C G A T A T C-5'

It is mutated to:

3'-T A C T T G T C C A A T A T C-5'

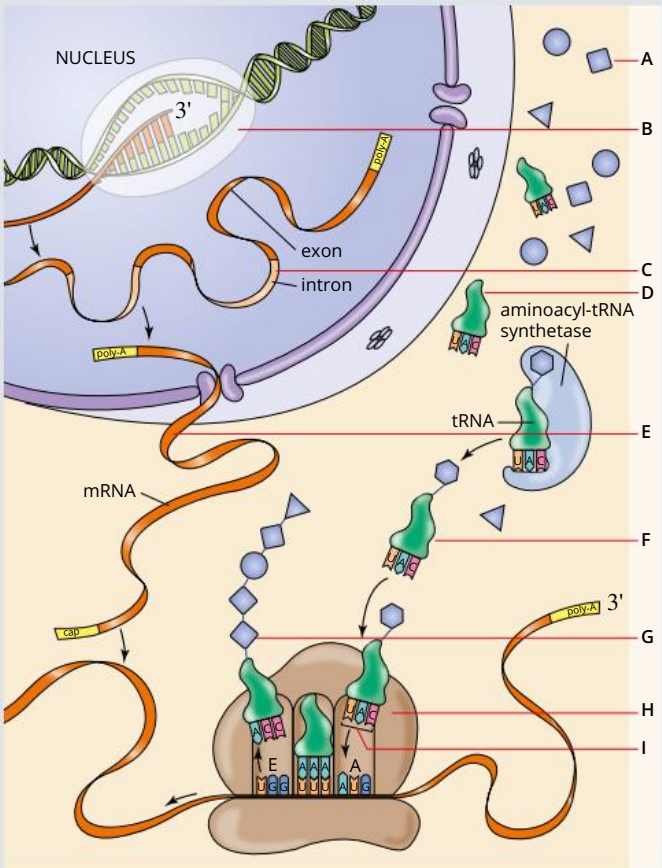
Justify the effect of the mutation on the amino acid sequence (using the translation table on page 276).

19 **a** Use the following terms to label the structures A–I on the diagram below:

- amino acid
- anticodon
- loaded tRNA
- mRNA
- polypeptide
- pre-mRNA (primary mRNA transcript)
- ribosome
- RNA polymerase
- unloaded tRNA

b Explain the role of structure B in protein synthesis.

c Explain the process occurring in structure H. Ensure you include the significance of structure I in your discussion.



20 Contrast splicing and alternative splicing in eukaryotic cells to explain how human cells can make 75 000–100 000 different proteins, given that there are about 20 000 genes.

21 Explain how somatic mutations differ in their effect on generations compared with germline mutations.

22 Use two examples to describe how a point mutation has led to significant phenotypic differences.

23 Complete the table below to show the differences between synonymous mutations, non-synonymous mutations, nonsense mutations and frameshift mutations.

Mutation type	Characteristic	Implications of mutation
synonymous		
non-synonymous		
nonsense		
frameshift		

24 Compare a spontaneous mutation to an induced mutation.

25 Explain the main function of homeotic genes and describe a consequence if a mutation occurs in one of these genes.

Analysis

26 Compare the gene expression processes of prokaryotic and eukaryotic organisms.

27 Determine the 5'–3' sequence of nucleotides in the DNA template strand for an mRNA coding for the polypeptide sequence Phe–Pro–Lys. Refer to Figure 5.2.8 on page 266.

A 5'-GAACCCCTT-3'
B 5'-AAAGGCTTT-3'
C 5'-CTTCGGGAA-3'
D 5'-AAACCCUUU-3'
E 5'-UUUGGGAAA-3'

28 A strand of nucleic acid is shown below.
AUG AAU CCU UAU GGU GGC UUU UAA
The protein produced as a result of the information encoded in the strand of nucleic acids is shown below.
Met–Asn–Pro–Phe

a Justify whether the strand given is DNA, pre-mRNA or mRNA.

b Determine the amino acid being transported by tRNA for translation of the nucleic acid sequence above with the anticodon to the second codon.

29 Outline why, in humans, the mature adult intestinal cell will produce digestive enzymes while endocrine gland cells produce hormones, even though both differentiated cells contain the same human genome and secrete proteins.

30 Justify Chargaff's rule from the data.

Nitrogenous base composition of various species of organisms

Source	Adenine (%)	Guanine (%)	Cytosine (%)	Thymine (%)
E. coli	24.7	26.0	25.7	23.6
wheat	28.1	21.8	22.7	27.4
sea urchin	32.8	17.7	17.3	32.1
salmon	29.7	20.8	20.4	29.1
human	30.4	19.6	19.9	30.1
ox	29.0	21.2	21.2	28.7

31 Predict the effect on protein synthesis of a mutation to the TATA box due to X-rays.

32 Predict the effect of a mutation in *E. coli* changing the *lac* operator so that the active repressor cannot bind, on the cell's production of β-galactosidase. Refer to the type of gene expression involved.

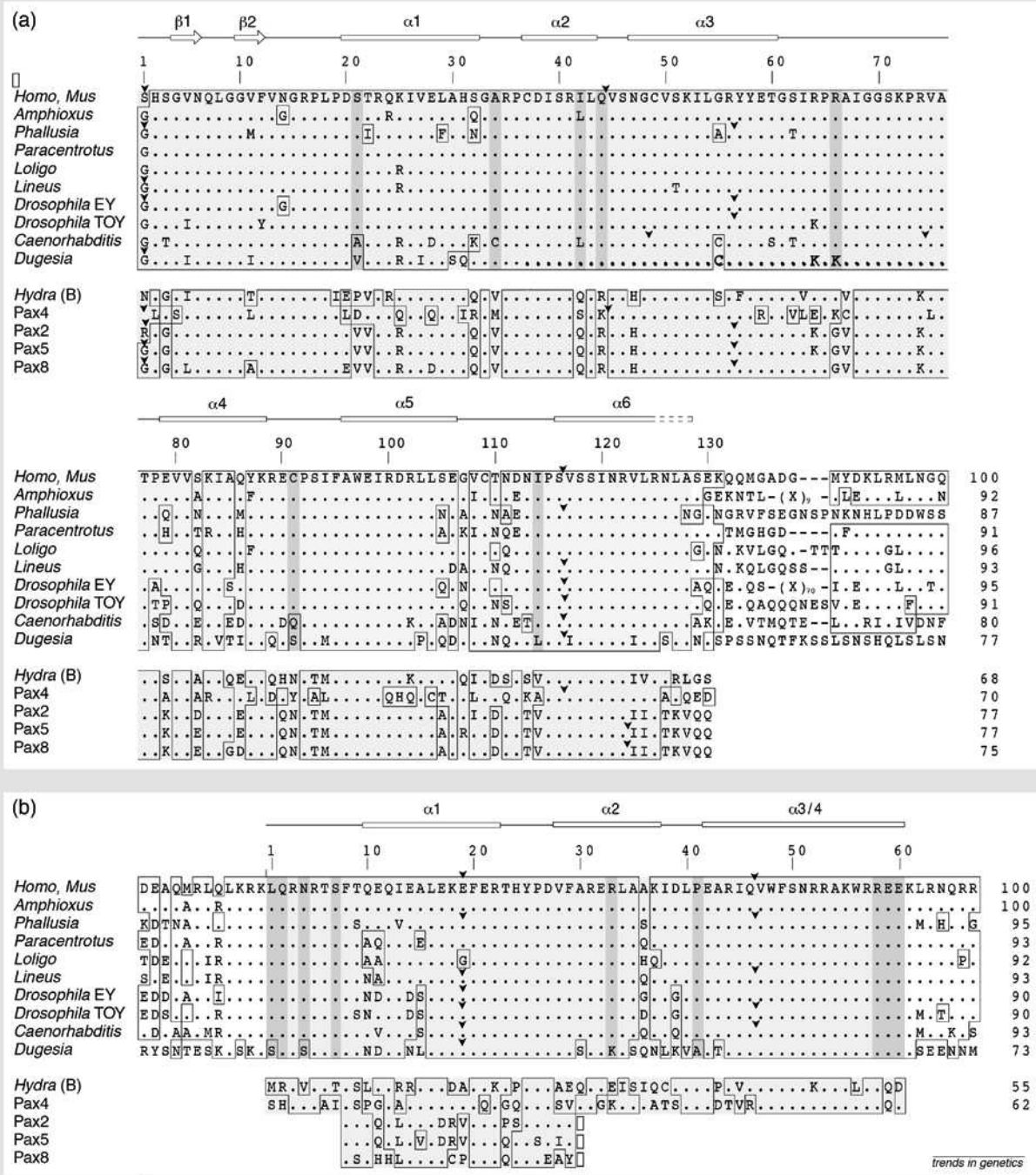
Knowledge utilisation

33 Argue which of the following mutations be would likely to cause the most harmful effect on an organism. Provide an evaluation of each.

- **a** a nucleotide-pair substitution
- **b** a deletion of three nucleotides near the middle of a gene
- **c** a single nucleotide deletion in the middle of an intron
- **d** a single nucleotide deletion near the end of the coding sequence
- **e** a single nucleotide insertion downstream of and close to the start of the coding sequence.

34 The *Pax6* gene encodes a transcription factor *Pax6* that regulates eye and lens development in different organisms. The protein consists of a sequence of 130 amino acids. An experiment was conducted to compare the amino acid sequence of this gene across a variety of vertebrate and invertebrate species. The figure on page 298 illustrates the results of the study. Different amino acids are represented by a different letter. The mammalian *Pax6* (human and mouse) sequence is provided on the first line and the analysis of the differences in the amino acid sequence was compared to this mammalian line. The numbers to the right of the diagram represent the percentage of amino acid sequence similarity with the mouse and human proteins.

- **a** Comment on the results of the experiment and propose to what group of genes *Pax6* belongs.
- **b** Identify any patterns in the differences between the *Pax6* amino acid sequence.
- **c** Discuss the evolutionary implications of the similarity between the *Pax6* transcription factors.
- **d** Predict the effect of manipulating the fruit fly genome to replace the *Pax6* gene with that from either the mouse or squid.



Key to species:
Homo—human
Mus—mouse
Loligo—squid
Drosophila—fruit fly
Paracentrotus—sea urchin
Caenorhabditis—nematode
Dugesia—flatworm

CHAPTER 06

Cellular replication and variation

In order to maintain the continuity of life, cells need to replicate (make copies of) themselves. This is the case for both unicellular and multicellular organisms.

In this chapter, you will investigate how eukaryotic and prokaryotic cells replicate, and the purposes of replication. You will also explore the production of gametes in sexual reproduction through the key events in meiosis. You will learn how sexual reproduction results in offspring with a set of unique characteristics that are inherited from their parents.

Syllabus subject matter

Topic 1 • DNA, genes and the continuity of life

DNA STRUCTURE AND REPLICATION

- explain the role of helicase (in terms of unwinding the double helix and separation of the strands) and DNA polymerase (in terms of formation of the new complementary strands) in the process of DNA replication. Reference should be made to the direction of replication.

CELLULAR REPLICATION AND VARIATION

- within the process of meiosis I and II:
  - recognise the role of homologous chromosomes
  - describe the processes of crossing over and recombination and demonstrate how they contribute to genetic variation
  - compare and contrast the process of spermatogenesis and oogenesis (with reference to haploid and diploid cells)
- demonstrate how the process of independent assortment and random fertilisation alter the variations in the genotype of offspring.

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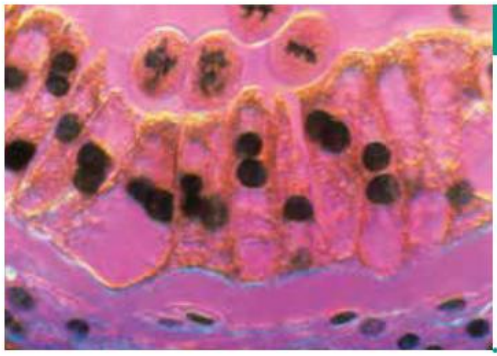
6.1 Cellular replication



BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- explain why cells replicate
- describe the cell cycle in terms of its main phases
- describe the process of binary fission in prokaryotic cells
- understand the process of DNA replication, including the role that the enzymes DNA helicase and DNA polymerase play in this process
- explain the directional structure of DNA, and contrast the synthesis of the leading strand and lagging strand.

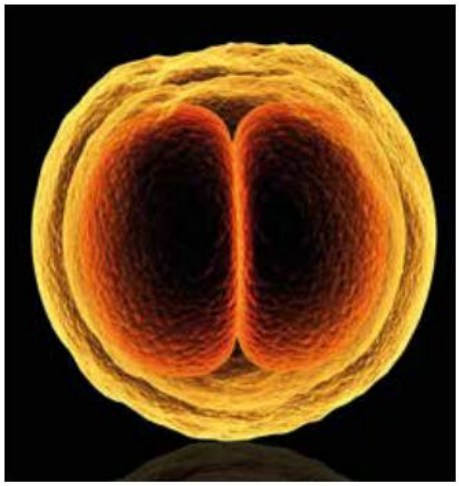


FIGURE 6.1.1 Coloured scanning electron micrograph of a fertilised human egg.

All people, including you, began life as a single cell. You were a fertilised egg cell, known as a **zygote** (Figure 6.1.1). Your body is now made up of about 37 trillion cells. To start creating the trillions of cells that make you, that first single cell had to replicate itself to become a fetus. As the cells in your body age and die, more cells are replicated to replace them. Replication results in genetically identical cells.

The cell theory states that all cells arise from pre-existing cells. For this to occur, cells must be able to replicate. This process is essential to the life of all organisms. In this module, you will learn the reasons for **cell replication** and the methods of replication for eukaryotes and prokaryotes. For cells to replicate, the DNA of the parent cell must also be replicated so that the two daughter cells will each have a complete set. In this module, you will also learn how copies of DNA are made.

CELL REPLICATION

Cell replication is a form of cell division in which a parent cell divides to produce two genetically identical daughter cells. It is essential that the genetic information is passed on accurately, because the activities of cells are controlled by the genetic information in the nucleus (in eukaryotes) or nucleoid (in prokaryotes).

Cell replication in prokaryotes

Prokaryotes, such as bacteria and cyanobacteria, are simple single-celled organisms (Figure 6.1.2). Prokaryotes have no nucleus or membrane-bound organelles. Instead, they have a single continuous (circular) DNA chromosome that may be anchored to the cell membrane at a point called the **origin**. Cell replication in prokaryotes occurs more quickly than in eukaryotes, because of the lack of organelles and the smaller amount of DNA in prokaryotes.

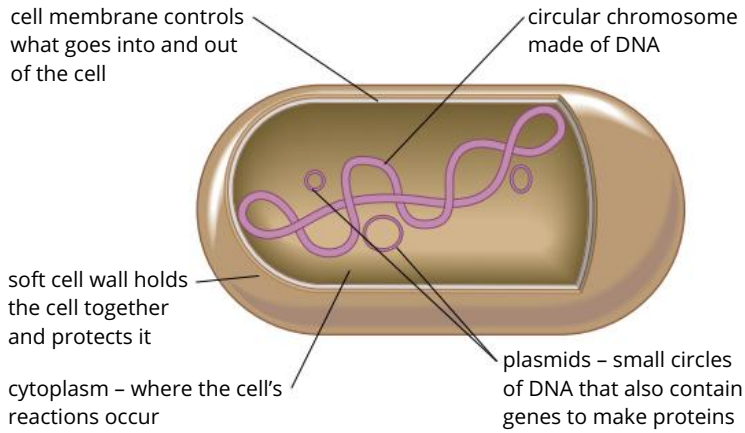


FIGURE 6.1.2 Prokaryotes are simple cells with a single continuous DNA chromosome.

Cell replication in prokaryotes is called **binary fission**. Binary fission is a relatively rapid form of reproduction that produces a new organism from the parent. Like mitotic division, binary fission is an exponential process because (in ideal conditions) the population doubles after every cycle of division. Binary fission proceeds in a sequence of steps (Figure 6.1.3). Replication of the chromosome begins at the origin, after which a new cell membrane and, where present, a new cell wall are produced to divide the parent cell. This results in the creation of two daughter cells.

Replication in prokaryotes is called binary fission. This is a rapid form of reproduction. The two daughter cells produced from a single parent cell are genetically identical.

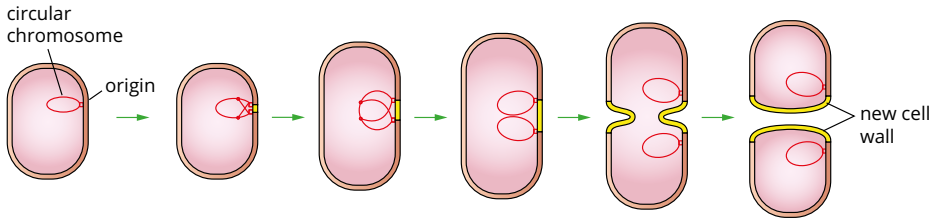


FIGURE 6.1.3 Binary fission of a single prokaryotic cell produces two daughter cells. Both daughter cells have a single DNA chromosome.

Some bacteria can undergo binary fission every 20 minutes. In other words, the number of cells can double every 20 minutes. In six hours, up to 18 cycles of binary fission could occur, allowing a single bacterium to produce 262 144 new cells (2^{18} = 262 144).

One consequence of binary fission is that (except for mutations) all organisms in a colony of bacteria are clones, or genetically identical (Figure 6.1.4). This means that a drug designed to kill a specific bacterium will kill all (or almost all) members of a bacterial colony that it comes into contact with.

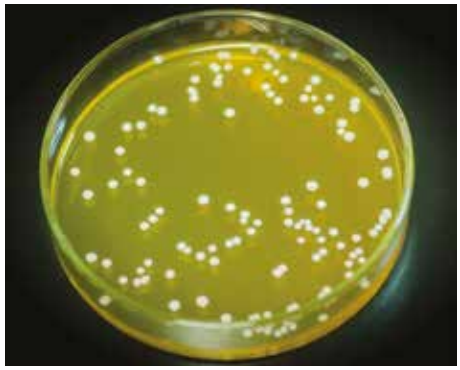


FIGURE 6.1.4 A colony of bacteria growing on an agar plate. Each bacterium in a colony is genetically identical.

Cell replication in eukaryotes

In eukaryotes (protists, fungi, plants and animals), cell replication involves a process called mitosis. Mitosis is part of the eukaryotic **cell cycle**. The cell cycle is the series of stages that a cell passes through, from its formation by cell division, through its growth and function, until it divides again. The cell cycle is described in detail later in this module.

Unicellular organisms remain a single cell throughout their entire life cycle, so their cells replicate for different reasons to cells in multicellular organisms. Cell replication in unicellular organisms (whether prokaryotes or eukaryotes) is a simple form of reproduction that creates a new, genetically identical individual.

In multicellular organisms, cells replicate for a number of reasons.

- As a cell grows, it can reach a point where there is too much cytoplasm for one nucleus to control. For example, after an egg is fertilised the cell divides repeatedly by mitosis to produce many smaller cells that restore the nucleus-to-cytoplasm ratio (Figure 6.1.5).

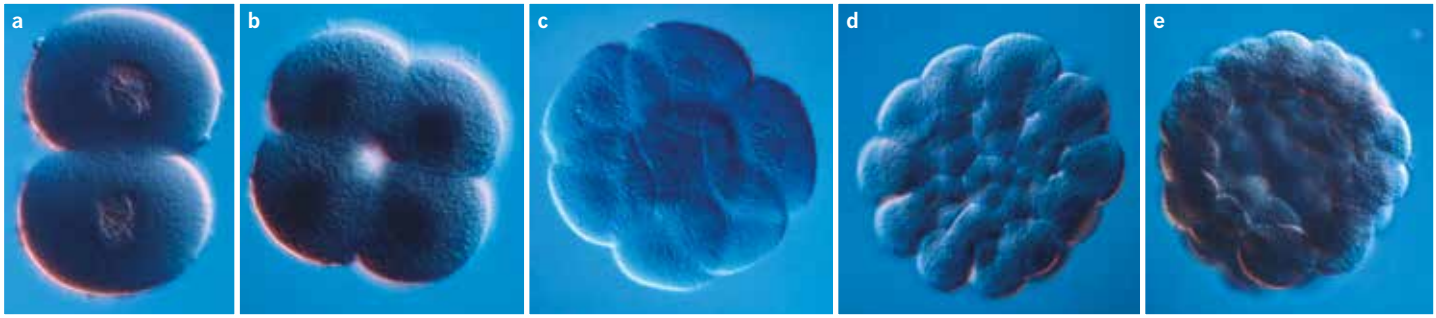


FIGURE 6.1.5 A series of light micrographs showing the change in the nucleus-to-cytoplasm ratio as a zygote divides. The first division (a) results in 2 cells; the second (b) results in 4 cells; the third (c) results in 8 cells; the fourth (d) results in 16 cells and the fifth (e) results in 32 cells.

Cell replication is a form of cell division in which a parent cell divides to produce two genetically identical daughter cells. In eukaryotes, cells replicate by mitosis to restore the nucleus-to-cytoplasm ratio, grow and develop, or maintain tissues and repair damage.

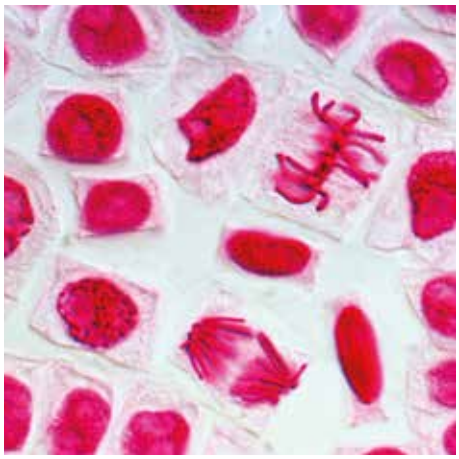


FIGURE 6.1.6 Light micrograph of hyacinth root at different stages of the cell cycle.

- Multicellular organisms grow by increasing the number of their cells through repeated cell replications. As the new individual continues to develop, new cells become specialised for different purposes.
- Cells in the tissues of multicellular organisms, such as the skin cells covering the body's surface, become damaged or die because of normal functioning or ageing, and also as a result of injury. In either case, maintaining and repairing tissues requires the production of new cells to replace those that die.

THE CELL CYCLE

Individual organisms, whether prokaryote or eukaryote, have a life cycle. A life cycle is simply the phases through which an organism passes during its life. For example, a butterfly's life cycle moves from egg to caterpillar to chrysalis to butterfly. The cycle of life begins again when the adult butterfly lays an egg.

Just like individual organisms, cells also have a life cycle. The phases in the life of a cell are known collectively as the cell cycle. The cell cycle begins with a single cell that grows and then divides into two daughter cells through replication. Figure 6.1.6 shows plant cells in different phases of the cell cycle.

The eukaryotic cell cycle

In eukaryotic cells, the cell cycle has three main phases. These phases always occur in the order described below.

1 Interphase: the cell doubles its mass and duplicates its entire components.
2 Mitosis: the nucleus divides.
3 Cytokinesis: the cytoplasm divides, marking the creation of the two new cells.

The cell cycle is the period between one cytokinesis and the next. In actively growing cells, mitosis and cytokinesis occupy only a small part of the cell cycle. Interphase occupies a large portion of the cell cycle (Figure 6.1.7).

The eukaryotic cell cycle is made up of three main phases: interphase, mitosis and cytokinesis.

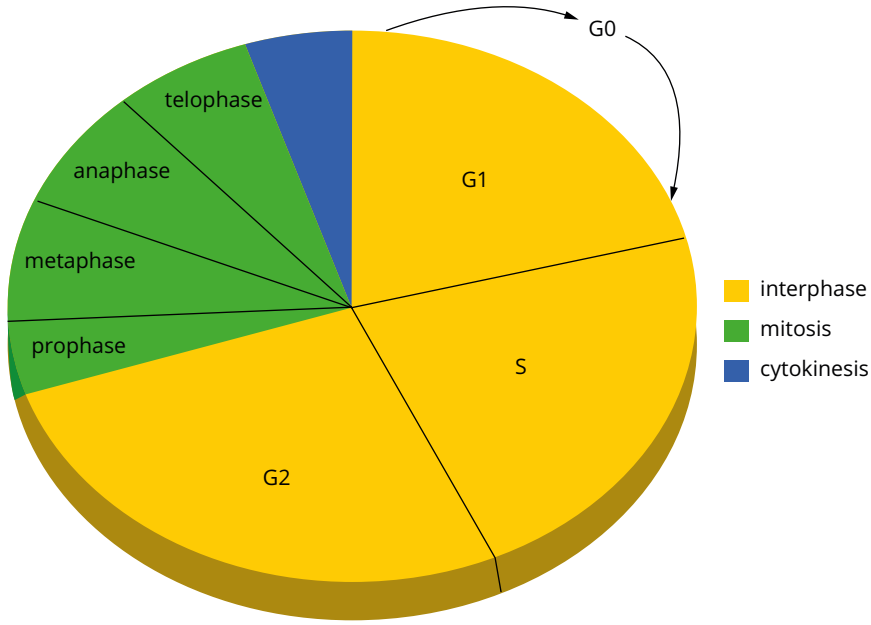


FIGURE 6.1.7 Mitosis and cytokinesis only make up a small proportion of the time taken for one cell cycle. Interphase occupies a much larger amount of time.

INTERPHASE

The first stage of the cell cycle is **interphase**. It begins immediately after the end of cell division. During interphase, a cell that is about to divide grows larger and copies its chromosomes in preparation for cell division.

Interphase is divided into three main phases:

1 G1 (pre-DNA replication)
2 S (DNA replication)
3 G2 (post-DNA replication).

The cell grows (G1), continues to grow as it duplicates its chromosomes (S), then grows more as it completes preparation for cell division (G2). As the cell grows, the normal functions of the cell continue, including the synthesis of the many components required for G1, S and G2.

There is sometimes a fourth phase called G0, also known as the 'resting phase'. The G0 phase is usually considered to be part of interphase. However, in diagrams, the G0 phase is sometimes drawn as an external section of the cell cycle to indicate that the cycle is not actively replicating organelles or DNA. This is because during G0, cells carry out the normal functions of the cell but do not change their internal structure or size. In most cells, G0 is temporary and ends when the cell re-enters the G1 phase. However, some specialised cells, such as nerve cells and red blood cells, remain permanently in the G0 phase and do not usually replicate.

The length of time a cell spends in interphase varies (Figure 6.1.8). Slow-growing cells, such as liver cells, spend many weeks or even years in interphase. Bone marrow cells may pass through interphase in less than a day as they generate many new blood cells. In an early embryo, there is little growth and the cells rush from one round of mitosis to the next. Interphase always lasts much longer than mitosis. Mitosis lasts for about two hours in a human cell.

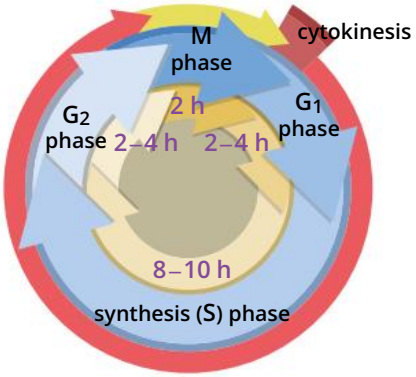


FIGURE 6.1.8 An example of the time phases (in hours) in a eukaryotic cell cycle. The S phase lasts 8 to 10 hours. The G1 and G2 second growth phases last for about 2 to 4 hours. The total cell cycle time in this example is between 14 and 20 hours.

DNA REPLICATION

DNA is passed on from the parent cell to the two daughter cells when the parent cell divides. To transmit an exact copy of the DNA, without losing any instructions, cells need a mechanism for accurately copying DNA. This mechanism occurs during the S phase of interphase. It is called **DNA replication**.

Most of the DNA in eukaryotic cells is located in the nucleus and is arranged in chromosomes. A chromosome consists of one long double-stranded DNA molecule (Module 5.1). During replication, the double strand separates into two template strands. A new complementary strand is synthesised onto each template strand (Figure 6.1.9).

> DNA replication is the process used by a parent cell to make an exact copy of its DNA. DNA replication is essential for cell division, as both daughter cells need a complete set of genetic information to function.

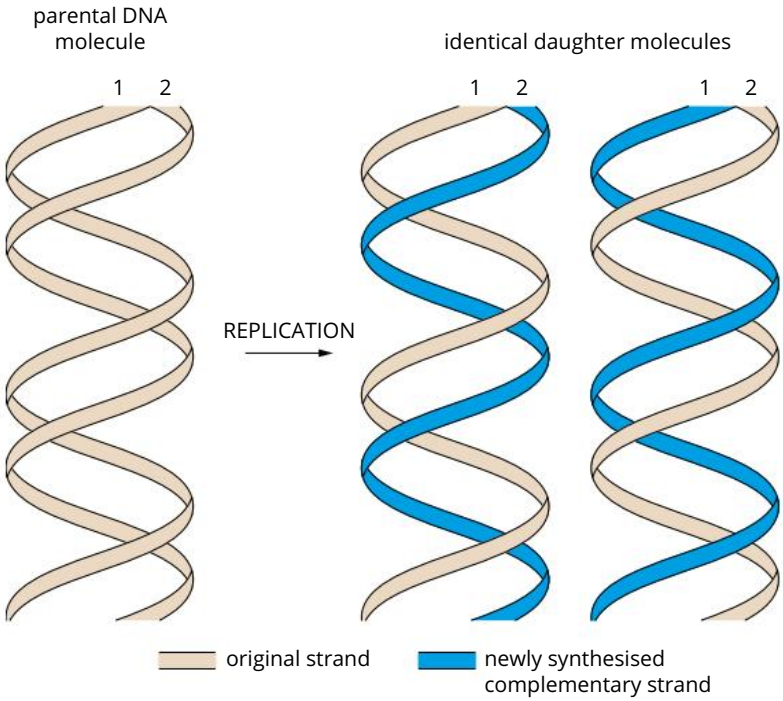


FIGURE 6.1.9 Each strand of the parent DNA molecule acts as a template for the synthesis of a new strand.

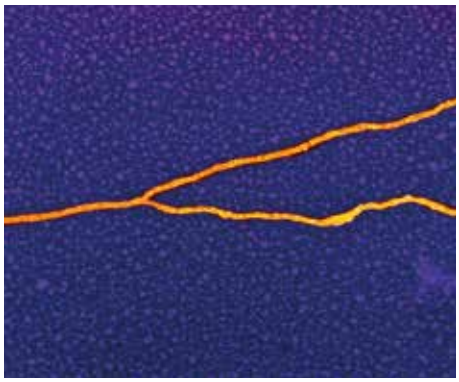


FIGURE 6.1.10 A coloured transmission electron micrograph of human DNA showing the replication fork.

Separation of the parent double strand of DNA into two template strands is achieved by an enzyme called **DNA helicase**. DNA helicase unwinds and then 'unzips' the parent DNA by breaking the hydrogen bonds between the complementary base pairs. As DNA helicase unzips the DNA, it forms a **replication fork** (Figure 6.1.10).

At the replication fork, a new polynucleotide chain is synthesised by adding complementary nucleotide units according to the sequence of the template strand. To make the new strand, new nucleotides pair up with the template strand, using the rules of complementary base pairing. A complementary strand is formed because adenine (A) pairs with thymine (T), and cytosine (C) pairs with guanine (G). Each daughter DNA molecule consists of one old strand from the parent and one newly synthesised strand, so DNA replication is described as **semi-conservative**.

The enzyme responsible for adding the nucleotides in the correct sequence is called **DNA polymerase**. DNA polymerases rarely add the wrong nucleotides, but if this does happen there are proofreading and repair enzymes to correct the error. Mutations only occur when these backup systems fail.

DNA replication is an extremely accurate process with three distinct phases (Figure 6.1.11):

1 The parent DNA molecule unwinds then 'unzips' and a replication fork forms, achieved by the action of an enzyme called DNA helicase.
2 Copying of the DNA proceeds, with complementary nucleotide bases (A–T and G–C) attaching to the parent strands. Nucleotides are added by an enzyme called DNA polymerase.
3 Replication results in two identical strands. One was the template strand and the other is a newly synthesised complementary strand.

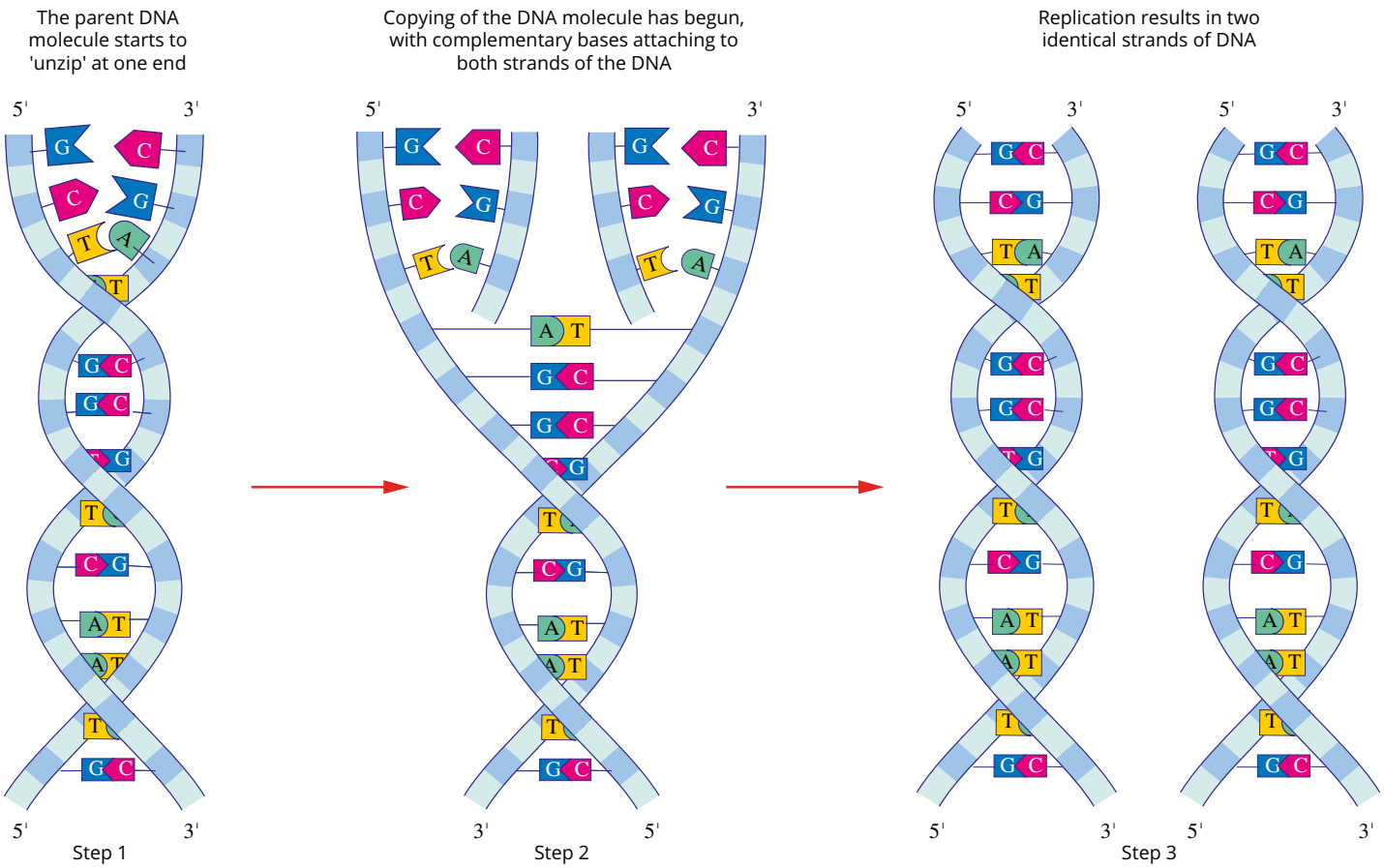


FIGURE 6.1.11 DNA replication involves three distinct phases.

Direction of DNA replication

As outlined in Chapter 5, the DNA molecule is made up of two antiparallel polynucleotide chains (strands). DNA polymerase can only add nucleotides to the 3' end of the growing strand. This means that one strand is being replicated in the same direction as the replication fork is moving. This strand is called the **leading strand** because its synthesis is less complex and occurs more quickly than its complement. The other strand is called the **lagging strand**. The lagging strand (with its orientation being antiparallel and the nucleotides being added to the 3' end) is synthesised in the opposite direction to the movement of the replication fork.

Once synthesis begins at the replication fork, the fork moves on. This leaves a gap on the lagging strand that requires another DNA polymerase to attach and begin replication again (Figure 6.1.12). Replication on the lagging strand is discontinuous and results in short sections of newly synthesised DNA. These are called **Okazaki fragments**, after their discoverer Dr Reiji Okazaki. The discontinuous fragments are eventually joined to produce a continuous strand of DNA.

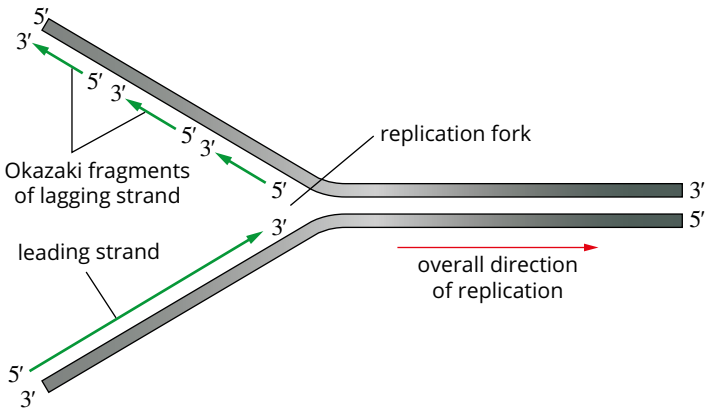


FIGURE 6.1.12 DNA replication showing the replication fork, the direction of replication and the leading and lagging strands. The lagging strand is made up of Okazaki fragments.

DNA replication is directional. The leading strand is synthesised in the same direction as the replication fork. The lagging strand is synthesised in the opposite direction, which results in small segments of DNA known as Okazaki fragments.

WS 4.1.5

6.1 Review

SUMMARY

- In eukaryotes (protists, fungi, plants and animals), cells replicate by mitosis.
- Cell replication in prokaryotes is called binary fission.
- In eukaryote cells, the cell cycle has three main phases:
  - interphase
  - mitosis
  - cytokinesis.
- The first stage of the cell cycle is interphase. During interphase, a cell that is about to divide grows larger and copies its chromosomes in preparation for cell division.
- Interphase is divided into three main phases:
  - G1 (pre-DNA replication)
  - S (DNA replication)
  - G2 (post-DNA replication).
- A fourth phase of the cell cycle is known as G0. During this phase, the cell carries out its normal cellular function; no cellular replication occurs.
- DNA replication is the mechanism used by a parent cell to make an exact copy of its DNA. This is required so that both of the two daughter cells have one complete copy of the DNA.
- During replication, each strand of parental DNA acts as a template onto which a new strand is synthesised.
- DNA replication has three distinct phases.
  - The parent DNA molecule unwinds then 'unzips', and a replication fork forms.
  - Copying of the DNA proceeds with complementary nucleotide bases (A–T and G–C) attaching to the parent strands.
  - Replication results in two identical DNA molecules.
- DNA polymerase is the enzyme responsible for adding the nucleotides in the correct sequence during replication.
- The replication fork forms when the complementary strands of the parent DNA 'unzip'. This separation is driven by an enzyme called DNA helicase.
- DNA replication is directional. The leading strand is synthesised in the same direction as the replication fork. The lagging strand is synthesised in the opposite direction. This results in short segments of DNA known as Okazaki fragments.

KEY QUESTIONS

Retrieval

1 List the three purposes of cell division in multicellular organisms.

2 Name the phase in the eukaryotic cell cycle where the cytoplasm divides.

3 State the phase of the cell cycle that is the longest.

4 Define DNA replication.

5 List the stages in the eukaryotic cell cycle.

6 Explain why cell replication occurs more quickly in prokaryotes than in eukaryotes.

Comprehension

7 Explain the role DNA helicase plays in the process of DNA replication.

8 Describe the key events that occur during binary fission in bacteria.

9 Explain why DNA replication is described as semi-conservative.

10 Classify the examples of cell replication into the correct purposes:

- a toddler's height increasing by 2 cm
- a cut healing
- a bacterium cell dividing
- an embryonic cell dividing
- a seed germinating
- a unicellular organism dividing

Purpose	Example(s)
restoring the nucleus-to-cytoplasm ratio	
growth and development	
repair and maintenance	
reproduction	

11 Explain the relationship between the time a cell spends in interphase and cell growth rate.

12 Describe how the DNA pattern of the parent cell is conserved accurately in the daughter cells.

13 Explain why replication of the lagging strand is different to that of the leading strand.

Analysis

14 Determine if unicellular organisms and multicellular organisms replicate for the same reasons. Explain your answer.

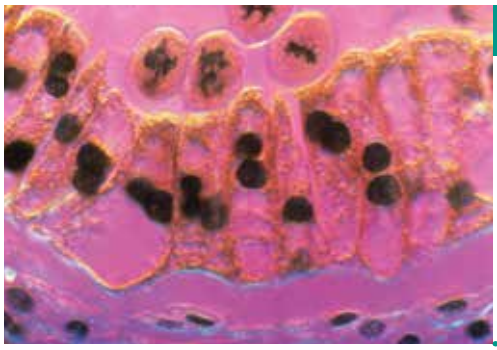
15 Explain, using binary fission, why antibacterial hand washes can kill up to 99.99% of bacteria.

16 If a bacterial organism undergoes binary fission every 30 minutes, calculate how many organisms there would be after 4 hours.

17 Not every cell goes through the G0 phase. Interpret this statement and explain the result if a cell does not move out of this phase.

18 Evaluate the accuracy of the following statement: 'It is necessary for two strands of DNA to run antiparallel to one another'.

6.2 Mitosis and cytokinesis



BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- describe the sub-phases of mitosis: prophase, metaphase, anaphase and telophase
- explain how identical copies of each chromosome are passed from the parent cell to two daughter cells during mitosis
- understand that cytokinesis is the division of the molecules and organelles of the cytoplasm, and the separation of the new nuclei, to form two new daughter cells.

There are three phases in the life cycle of a eukaryotic cell. The first phase is called interphase, which was described in detail in the previous module. Typically, a cell spends most of its time in interphase. Following interphase, the cell moves into mitosis. It is during mitosis that division of the nucleus occurs. The final phase in the cell cycle is cytokinesis. During cytokinesis the cytoplasm divides, creating two new cells.

In this module, you will learn about the phases of mitosis and cytokinesis.

MITOSIS

Mitosis is the part of eukaryotic cell cycle when division of the nucleus occurs. During mitosis, identical copies of each chromosome are passed from the parent cell to two daughter cells. The result of mitosis is two daughter cells that have identical genetic material as the parent cell. Thus, a diploid ($2n$) parent cell that undergoes mitosis will produce two diploid ($2n$) daughter cells.

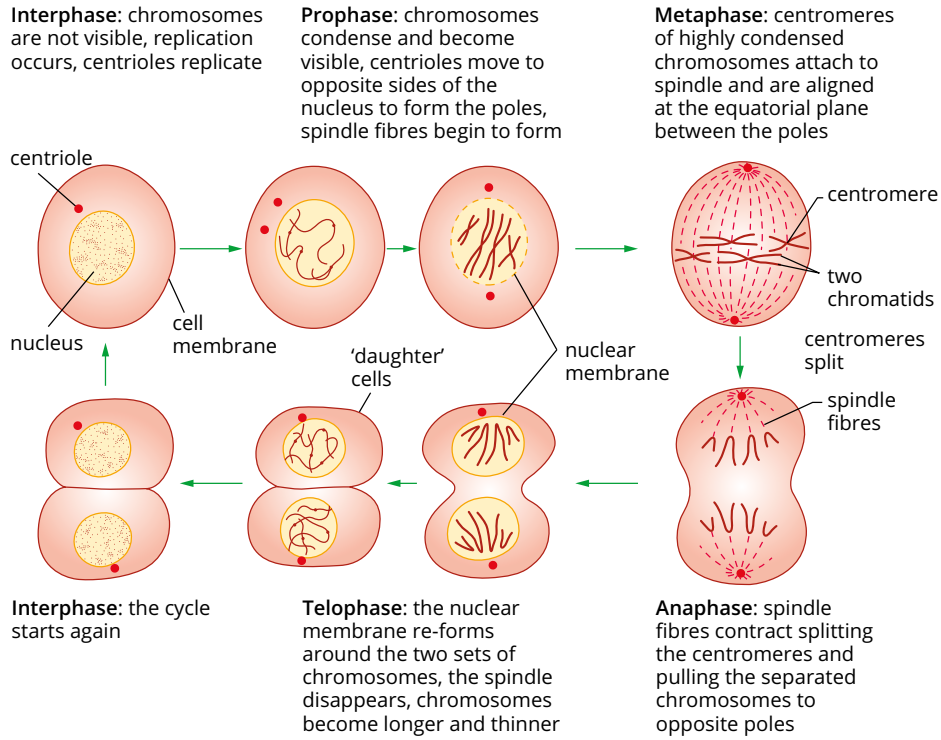


FIGURE 6.2.1 The movement of chromosomes during the four major stages of mitosis.

Mitosis a continuous process with four sub-phases:

- prophase
- metaphase
- anaphase
- telophase.

Each sub-phase can be distinguished by the appearance and position of the chromosomes in the cell (Figure 6.2.1).

Prophase

Early in **prophase**, chromosomes begin to condense (shorten and thicken) and become increasingly visible under the microscope. As they condense, each chromosome can be seen as two **chromatids** held together at the **centromere** (Figure 6.2.2). At the same time, the **centrioles**, which were replicated during interphase, move to opposite ends of the cell to form the poles.

Later in prophase, the nuclear membrane breaks down. The centrioles begin to form a network of fibres, called **spindles**, which extends between the two poles of the cell. The centromere of each individual chromosome attaches to spindle fibres extending from each of the poles. Plant cells do not usually have centrioles; they use a different mechanism to produce the mitotic spindle.

FIGURE 6.2.2 Coloured scanning electron micrograph of a human chromosome that has replicated during cell division. It now consists of two identical chromatids linked at their centre by a centromere.

Metaphase

During **metaphase**, the centromeres continue to be drawn by the spindle fibres so that the chromosomes are aligned in the middle of the cell. Chromosomes are most easily observed at this stage because they are highly condensed.

Anaphase

In **anaphase**, the spindle fibres contract, pulling the centromere in two directions. The centromere splits, separating the two chromatids. The spindle fibres continue to contract and the separated chromatids are pulled to opposite poles. The resulting two daughter cells each receive the same genetic information: one copy of every chromosome that was in the original nucleus at interphase.

Telophase

The final sub-phase of mitosis is called **telophase**. A nuclear membrane forms around the chromosomes at each pole. The spindle is dismantled and disappears. The chromosomes become longer and thinner, and are less visible under the microscope.

At the end of mitosis, each new nucleus contains one copy of every chromosome that was present in the parental nucleus. Interphase and the four sub-phases of mitosis are shown in Figure 6.2.3.

i Division of the nucleus occurs in the mitosis phase of the eukaryotic cell cycle. Mitosis can be divided into four sub-phases: prophase, metaphase, anaphase and telophase.

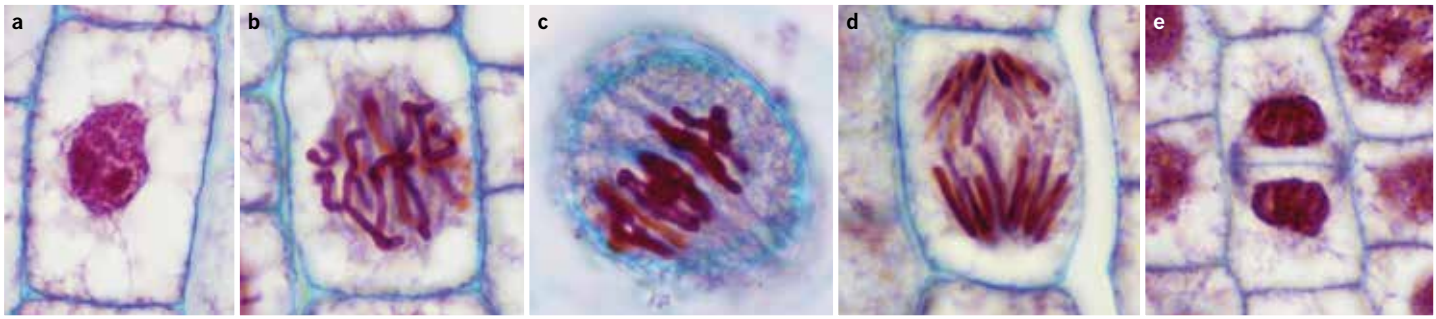


FIGURE 6.2.3 Light micrographs of mitotic division in a garlic root tip cell: (a) interphase (not part of mitosis), (b) prophase, (c) metaphase, (d) anaphase and (e) telophase.

CYTOKINESIS

The final stage of the eukaryotic cell cycle is **cytokinesis**. During cytokinesis, the cytoplasm divides and the two new nuclei separate (Figure 6.2.4). The division of the cytoplasm during cytokinesis marks the creation of the two new cells.

Cytokinesis in animal cells is different to cytokinesis in plant and fungi cells. In animal cells, the cell membrane moves inwards, pinching the two daughter cells apart. Unlike animal cells, plant and fungi cells have a cell wall that must be created around each new cell. Plant and fungi cells have to lay down a new cell membrane and cell wall between the two daughter nuclei to separate the daughter cells. Components of the new cell wall, called the **cell plate**, are initially deposited in the centre of the cell (Figure 6.2.5). The growth of the cell plate extends outwards until the two daughter cells are completely separated.

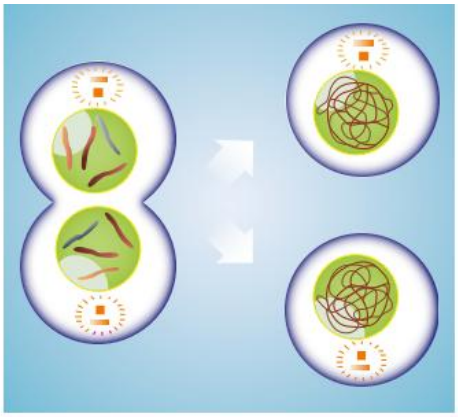


FIGURE 6.2.4 Cytokinesis results in the creation of two daughter cells. The cytoplasm of the parent cell is divided into two by the cell membrane.

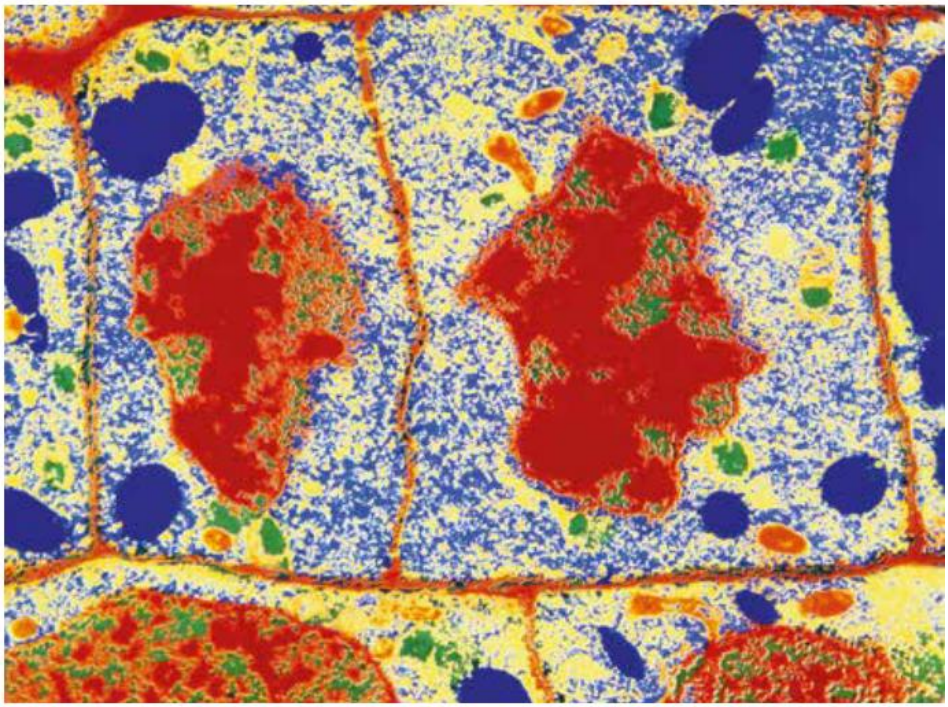


FIGURE 6.2.5 A coloured transmission electron microscopy image of the late stage of cell division in a plant cell. The daughter nuclei (red and green bodies) are re-forming into membrane-bound organelles. Between the two forming nuclei is the developing cell wall.

In some cells, cytokinesis is not always completed during mitosis. This results in a large cell that contains many nuclei. This type of cell is called a **coenocyte**. Examples of coenocytes include the endosperm of plant seeds, filamentous fungi and plasmodial slime moulds. In contrast, a **syncytium** is a multinuclear cell formed from fusion of many separate cells, rather than a lack of cytokinesis. The absence of cytokinesis is not the only way in which large cells with multiple nuclei form. For example, skeletal muscle is composed of bundles of multinucleated fibres that form from the fusion of mononucleated muscle cells known as myocytes.

> The absence of cytokinesis is not the only way in which large cells with multiple nuclei form. Skeletal muscle is an example of the fusion of myocytes (cells found in muscle tissue) that arises through normal mitotic divisions.

6.2 Review

SUMMARY

- Mitosis is a continuous process with four sub-phases: prophase, metaphase, anaphase and telophase.
- During mitosis, identical copies of each chromosome are passed from the parent cell to two daughter cells.
- Cytokinesis is the division of the molecules and organelles of the cytoplasm, and the separation of the new nuclei, to form two new daughter cells.
- The characteristics of the sub-phases of mitosis are summarised in the table below:

The characteristic sub-phases of mitosis	
prophase	• Chromosomes condense and become visible. • Centrioles move to opposite sides of the nucleus and form poles. • The nuclear membrane breaks down. • Centrioles form spindle fibres between the two poles. • The centromere of each chromosome attaches to spindle fibres extending from each pole.
metaphase	• Chromosomes align at the equatorial plane of the cell. • Spindle fibres attach to the centromeres of the chromosomes.
anaphase	• Spindle fibres contract, splitting the centromeres and separating the sister chromatids. • The single-strand chromosomes are separated. • Chromosomes are pulled to opposite poles.
telophase	• The nuclear membrane re-forms around the two sets of chromosomes. • Spindle fibres disappear. • Chromosomes become longer and thinner.

KEY QUESTIONS

Retrieval

1 Identify the sub-phase of mitosis in which the spindle fibres contract, pulling the centromere in two directions.

2 Define mitosis.

Comprehension

3 For a cell that has a diploid number of 8, determine the number of chromosomes present in a cell during:

a interphase

b prophase

c anaphase.

4 Describe the process of cytokinesis.

5 Use diagrams to show the events that occur at each phase of mitosis.

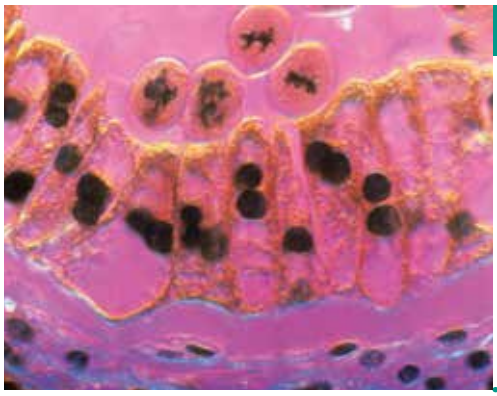
6 Explain the role that spindle fibres play in mitosis.

Analysis

7 Contrast cell division in plants with cell division in animals.

8 Compare a coenocyte to a syncytium.

6.3 Meiosis



BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- explain the role of meiosis in sexual reproduction
- understand that somatic cells are diploid and gametes are haploid
- describe the stages of meiosis and compare cell meiotic and mitotic cell division
- explain the role of homologous chromosomes in meiosis
- explain the process of crossing over and how it contributes to genetic variation
- compare and contrast the processes of spermatogenesis and oogenesis.

FIGURE 6.3.1 Blue ringtail damselflies form a 'wheel' when mating. The male (top) is holding the female's neck, while the female has moved her abdomen towards the male's genitalia to receive his sperm.

Sexual reproduction involves the union of male and female sex cells to form a unique individual (Figure 6.3.1). Most multicellular organisms, and some unicellular organisms, can reproduce sexually.

In this module, you will learn about the biological advantages of sexual reproduction and why an offspring of two parents has a unique genetic identity. You will also explore the key events in meiosis that result in the production of **gametes** from somatic cells.

GAMETES AND SEXUAL REPRODUCTION

Multicellular organisms are composed of two main types of cells: somatic cells and germ cells. **Somatic cells** are all the cells in the body of an organism apart from the sex cells (gametes). Examples of somatic cells include skin cells, muscle cells and nerve cells. **Germ cells** are the cells that give rise to gametes. Gametes are the specialised sex cells that combine in sexual reproduction. Examples are sperm cells in the testes and oocytes (i.e. egg or ova) in the ovaries.

The formation of gametes occurs by meiosis. This process takes place in specialised reproductive organs, called gonads. A normal eukaryotic organism is composed of diploid ($2n$) cells. Diploid cells have two sets of chromosomes: one set from each parent. Gametes formed by meiosis are haploid (n) as they only have one set of chromosomes.

Male and female gametes are often different in appearance. Female gametes (eggs or ova) lack motility. They are large cells that contain the food stores and cellular machinery needed for the development of the embryo. Male gametes (sperm or **spermatozoa**) contain limited food reserves and usually have a tail (or flagellum) for motility. This helps them move towards an egg.

Fertilisation occurs when two haploid cells fuse to form a diploid zygote (Figure 6.3.2). It involves many processes and at the point when the two sets of chromosomes unify to direct the zygote's survival and processes, a new individual has been conceived.

The zygote undergoes mitosis, dividing repeatedly to produce a large number of genetically identical cells (Module 6.1). These cells differentiate to form the tissues that make up the new organism. The organism continues to develop by mitotic divisions and becomes a mature adult. The reproductive cycle may then begin again.

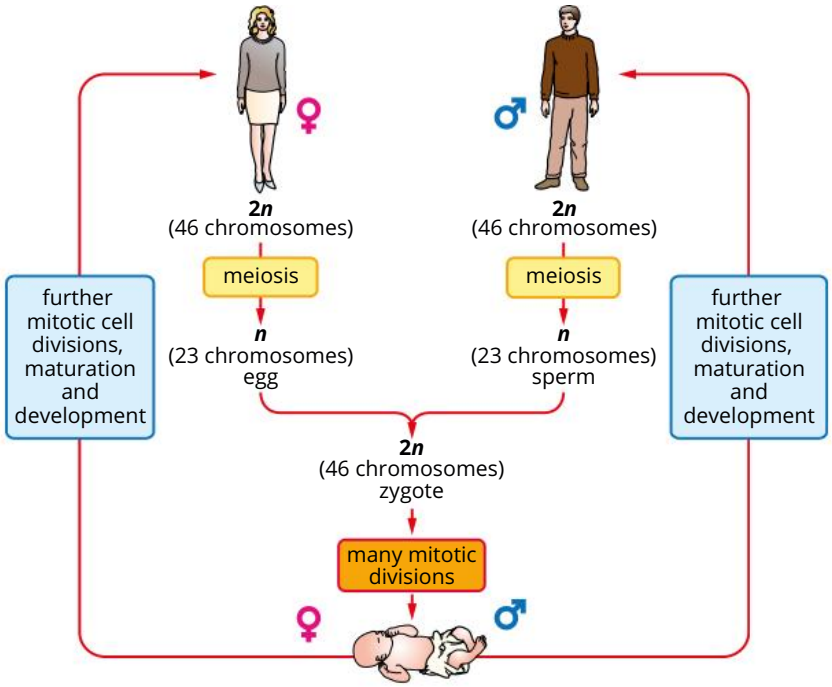


FIGURE 6.3.2 Meiotic cell divisions in females and males give rise to haploid (*n*) eggs and sperm, respectively. When fertilisation occurs, an egg and a sperm fuse to form a diploid (2*n*) zygote. The zygote develops into a new organism after many mitotic divisions and cellular differentiation.

MEIOSIS PRODUCES GAMETES

The process of cell division that produces gametes is called **meiosis**. Meiosis occurs only in eukaryotes, and only in the germ cells. Meiosis is essential for sexual reproduction and the creation of genetic variation. Although it is similar to mitosis, the outcomes of meiosis are very different (Figure 6.3.3). Interphase is equivalent before meiosis begins, but the genetic events in **meiosis I** are significantly different to those of mitosis. Also, there is no interphase between meiosis I and **meiosis II**, meaning meiosis II does not resemble mitosis except the sequence of stages.

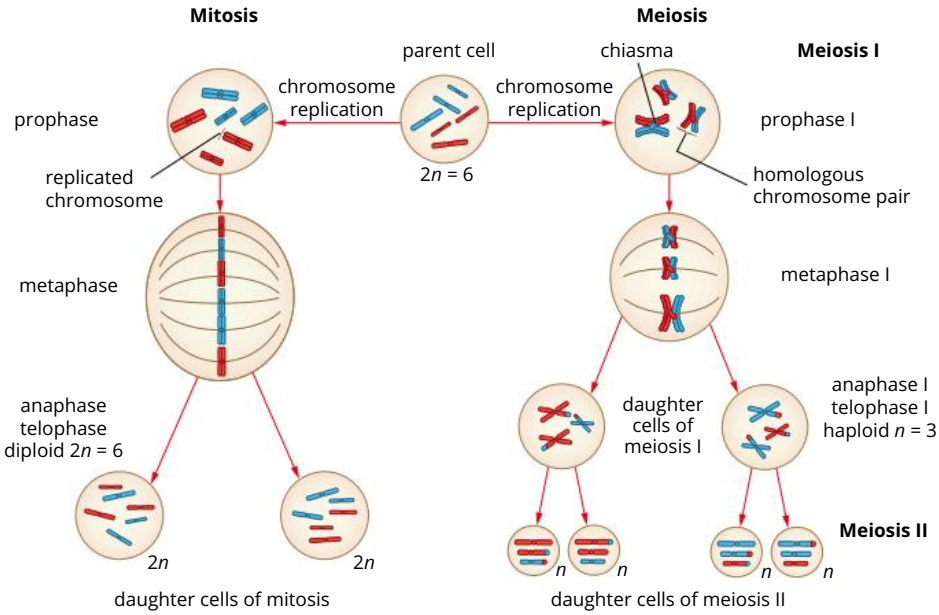


FIGURE 6.3.3 Mitosis and meiosis are both processes of cell division, but they are different in a number of important ways.

Figure 6.3.3 (page 313) and Table 6.3.1 highlight the key differences between mitosis and meiosis.

TABLE 6.3.1 Key differences between mitosis and meiosis

	Mitosis	Meiosis
number of cells	Mitosis results in two genetically identical daughter cells.	Meiosis produces four genetically unique daughter cells.
number of chromosomes	The daughter cells produced from mitosis are diploid (2*n*). They have the same number of chromosomes as the parent.	The daughter cells produced from meiosis are haploid (*n*). They have half the number of chromosomes of the parent.
genetic recombination	Mitosis does not involve recombination of alleles.	Meiosis rearranges alleles between chromosome pairs, creating unique genetic variation.

Meiosis produces haploid gametes

To maintain the chromosomal number of a species, the number of chromosomes in somatic cells and gametes is different. In humans, somatic cells have 46 chromosomes (23 pairs) and gametes have 23 chromosomes. When two gametes combine during fertilisation, the resulting zygote will have a full complement of chromosomes (23 + 23 = 46).

Somatic cells are diploid (2*n*) because they have gained one chromosome from each parent. These matching pairs of chromosomes are called **homologous chromosomes**. An individual inherits one set of each chromosome, with matching genes, from each parent. The homologous chromosomes carry the same genes, meaning that an individual inherits two copies of every gene, although the alleles may be different.

Meiosis includes two sequential rounds of division: meiosis I and meiosis II. These divisions enable the resulting gametes to each have the correct haploid number of chromosomes.

During meiosis I, the homologous chromosomes are separated. This reduces the chromosome number by half (reduction division) and produces two haploid daughter cells, each with a single set of chromosomes with two sister chromatids. In meiosis II, the two sister chromatids that make up each chromosome are separated to produce four haploid daughter cells.

Stages of meiosis

Like mitosis, meiosis is a form of cell division that has four sub-phases: prophase, metaphase, anaphase and telophase (Figure 6.3.4). Each sub-phase occurs in both meiosis I and meiosis II. The stages of meiosis occur in the following order:

1 Prophase I: Chromosomes condense from fine threads and spindle forms. Each chromosome is composed of two chromatids, connected at the centromere. **Crossing over** of homologous chromosomes occurs in late prophase.

2 Metaphase I: Homologous chromosomes pair up and align on the equatorial plate, chromatids (two per chromosome) become apparent, and the nuclear membrane breaks down. The homologous pairs are independently assorted along the equatorial plate. In other words, each side of the equatorial plate will have a random number of maternal and paternal chromosomes for each of the homologous pairs.

3 Anaphase I: The spindle draws members of each chromosome pair to opposite poles of the cell, and the cell membrane begins to pinch in.

4 Telophase I and cytokinesis: The cytoplasm divides and nuclear membranes form. Two haploid daughter nuclei are created, but each chromosome is still in the replicated state.

In meiosis, two successive cell divisions produce four daughter cells with half the number of chromosomes of the parent cell and new genetic variation.

5 Prophase II: The nuclear envelope breaks down and the meiotic spindle is re-created.
6 Metaphase II: Chromosomes at each end of the cell move to a central position. This begins the second division.
7 Anaphase II: The centromeres split, separating the sister chromatids and the single-strand chromosomes move to opposite poles of the cell. This results in haploid cells.
8 Telophase II and cytokinesis: The cytoplasm divides and nuclear membranes re-form. Four haploid daughter cells are created with different genetic combinations of the original set of homologous chromosomes.

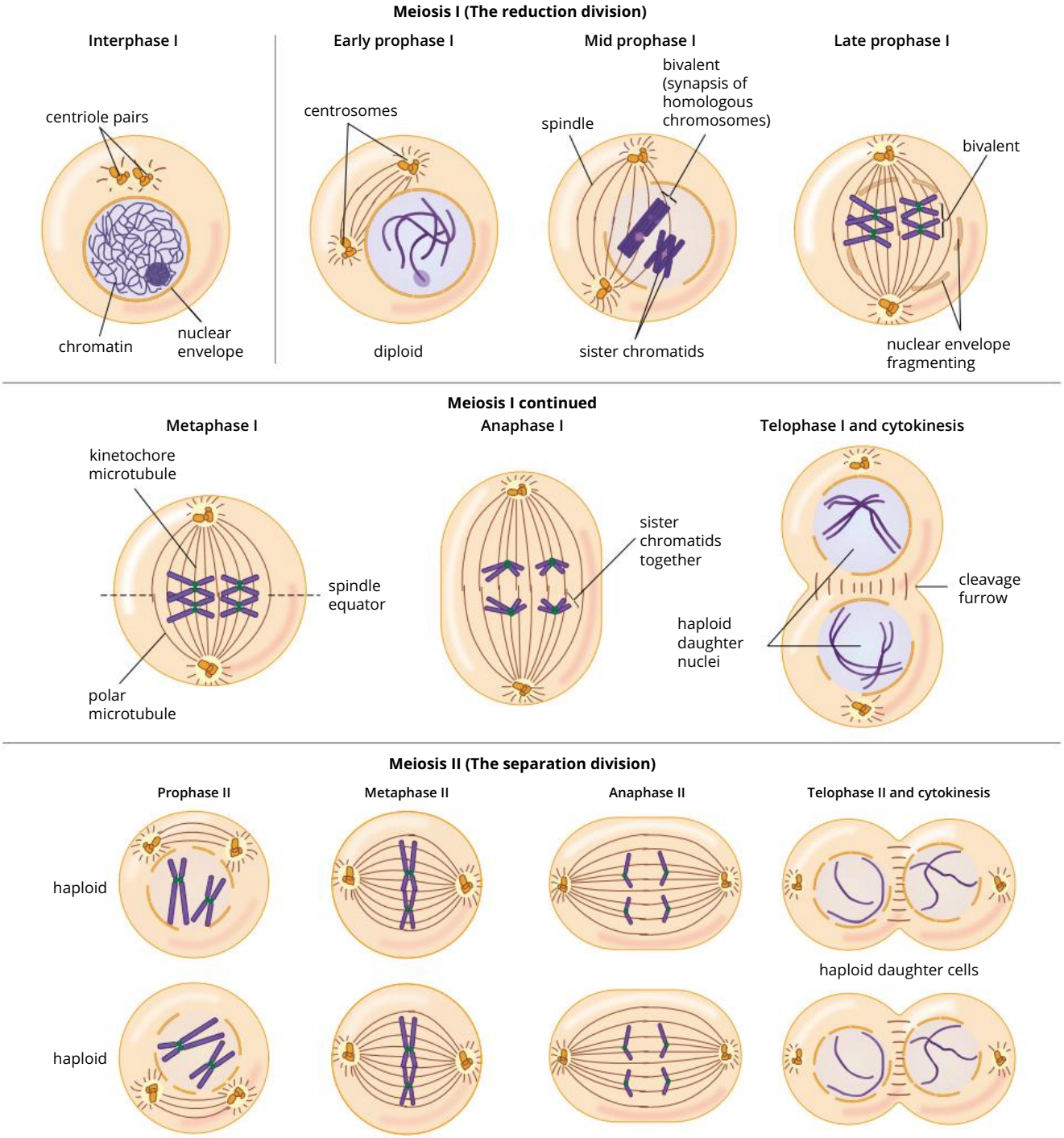


FIGURE 6.3.4 Stages of meiosis in a diploid (2*n*) animal cell. Reduction in the chromosome number, crossing over and genetic recombination occur in the first meiotic division. By the end of the second division, four haploid (*n*) nuclei have been produced from the one original parent cell.

FIGURE 6.3.5 A light micrograph showing the formation of chiasmata (circled) between homologous chromosomes.

Crossing over

During prophase 1, a significant event that introduces genetic variation may occur. Chromatids of homologous chromosomes may exchange portions of their genetic information in a process called crossing over. Crossing over is a natural genetic process that occurs between homologous chromosomes and leads to genetic material being swapped between the chromosomes. DNA strands from the chromatids of two homologous chromosomes are cut at an equivalent point, a segment is exchanged and the strands are reconnected (Figure 6.3.5). Paternal segments of DNA end up on the maternal chromosome, and maternal DNA segments end up on the paternal chromosome.

The point where crossing over occurs is called a **chiasma** (plural chiasmata). It consists of a temporary molecular scaffold, which disappears later. A long chromosome may have several chiasmata.

The significance of crossing over is that it produces chromosomes that have brand new combinations of genetic information. This contributes to genetic variation. This process is called **recombination**.

When crossing over is finished, the homologous chromosome pairs align along the midline of the cell. They do this randomly, meaning the maternal and paternal chromosomes do not line up on the same side of the midline. The homologues then separate and move to opposite poles. These two steps result in the independent assortment of maternal and paternal chromosomes and their alleles in the gametes. The centromeres do not split. It is the chromosomes of a pair that separate, not the chromatids. The spindle breaks down and the nuclear membrane may re-form.

At the end of meiosis I, there are two daughter cells with the chromosome number halved. Each daughter cell contains only one set (n) of chromosomes. Each chromosome is still made up of two chromatids.

The likelihood of two genes on the same chromosome exchanging alleles is related to the distance between these genes. The further apart the genes are, the greater the probability that crossing over will occur.

Sexual reproduction results in genetic variation

Each gamete has its own unique combination of alleles. There will be similarities in genetic content between parents and offspring, but the offspring are always genetically different from their parents and offspring have their own genotype (the set of alleles present in their DNA) (Figure 6.3.6). The concept of genotype will be discussed in detail in Chapter 7. This genetic variation arises from two features of meiosis.

FIGURE 6.3.6 The physical differences between family members are due to the unique combination of genes that are packaged into gametes during meiosis, and the recombination of this genetic material during fertilisation.

1 When chromosomes pair up during meiosis, crossing over produces chromosomes with new combinations of genetic information. Each gamete has a unique combination of alleles.
2 Gametes have only half the genetic information of their parent cell, because they receive only a single set of chromosomes from the parent cell. During metaphase I, the pairs of homologous chromosomes line up at the equator of the cell. Which side each homologue lines up on, and therefore which pole it will be pulled towards in anaphase I, is an independent event. Each gamete will have a random set of homologues in a process that results in independent assortment. Independent assortment is covered in detail in Chapter 7.

Meiosis ensures that a wide range of genetic combinations occurs during the formation of gametes. In humans there are 2^{23} combinations possible for sets of homologues in meiosis I. Variability is further increased when different genetic combinations are brought together at fertilisation. For humans this is: 2^{23} (egg or ova) $\times$ 2^{23} (sperm) possible combinations, which equals 2^{46} possible genetic variations (not including crossing over). Populations of organisms that reproduce sexually have considerable genetic variation. This enables the species to survive and reproduce in varied and changing environments.

Every person is unique because of the genetic variation created during gamete formation (meiosis) and recombination that occurred at their conception.

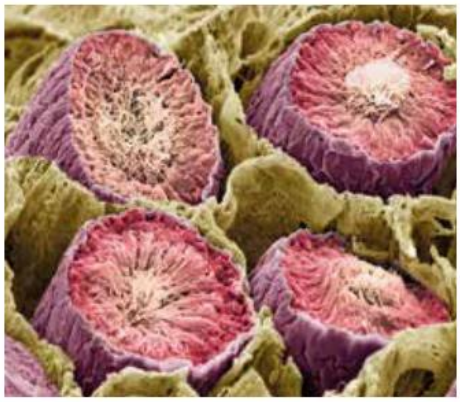


FIGURE 6.3.7 A coloured scanning electron micrograph of sperm cells in the seminiferous tubules of a testis. This is where sperm are produced and mature.

SPERMATOGENESIS AND OOGENESIS

A full complement of human chromosomes is 46. These 46 chromosomes are made up of 22 pairs of **autosomes** and one pair of **sex chromosomes** (**allosomes**). The allosomes are the sex-determining chromosomes. In humans, these are called X and Y chromosomes. In human females, the sex-determining chromosomes are a homologous pair of X chromosomes (XX). In human males, they are one X and one Y chromosome (XY). The presence of a Y chromosome determines male development.

Spermatogenesis

After meiosis in males, four haploid sperm are formed from the original diploid parent cell. Each sperm cell contains 23 chromosomes (one of each autosome and either an X or Y chromosome). The production of sperms is called **spermatogenesis**. Spermatogenesis begins with the mitotic divisions of precursor spermatogonium cells to produce **spermatocytes**. In humans, this occurs in the seminiferous tubules of the testes (Figure 6.3.7).

Spermatocytes are diploid. A single spermatocyte divides by meiosis to produce four haploid spermatid cells. Spermatids are nourished by special cells, called Sertoli cells. When the spermatids mature, they become spermatozoa. Spermatozoa (singular spermatozoon) is the term given to motile sperm cells that possess a flagellum. Figure 6.3.8 shows the process of spermatogenesis.

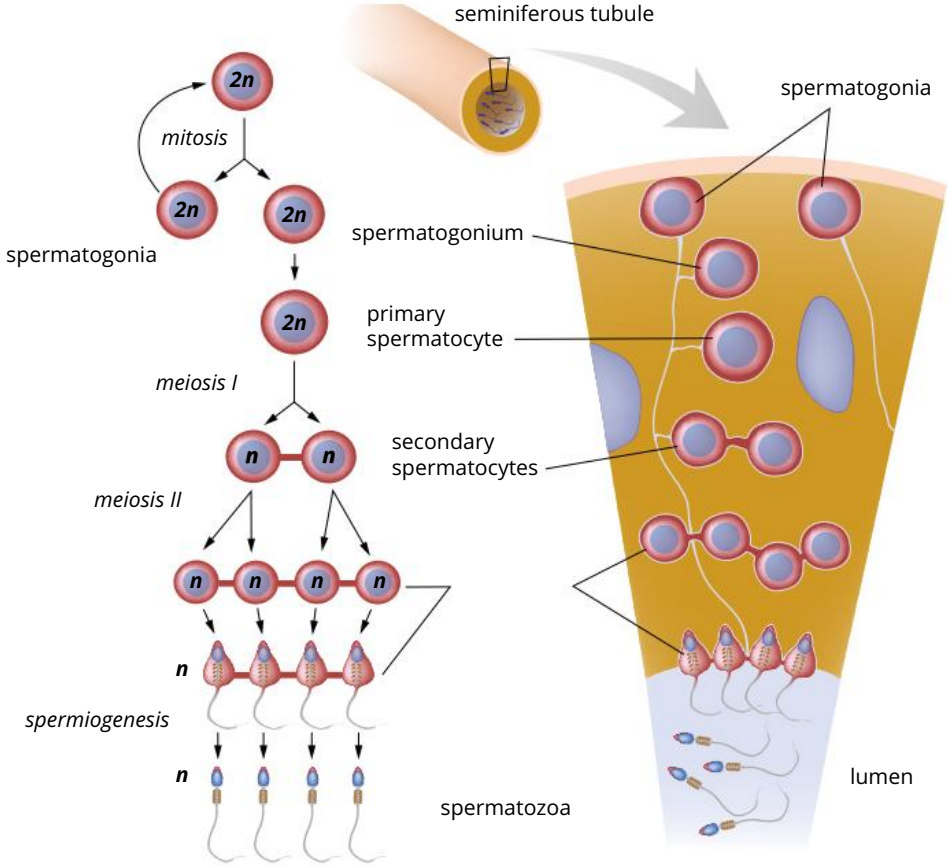


FIGURE 6.3.8 The process of spermatogenesis. Precursor cells, called spermatogonia, divide mitotically to produce spermatocytes. Spermatocytes are diploid and divide meiotically to produce haploid spermatozoa.

Oogenesis

In females, meiosis produces only one viable haploid cell over a series of several events. The three other haploid cells, which are called polar bodies, do not develop into viable ova because of the uneven distribution of cytoplasm in cytokinesis. One daughter cell is very large and contains most of the cytoplasm. The three polar bodies degenerate (Figure 6.3.9). Nevertheless, all four haploid cells contain 23 chromosomes (one of each autosome and one X chromosome) as outlined above.

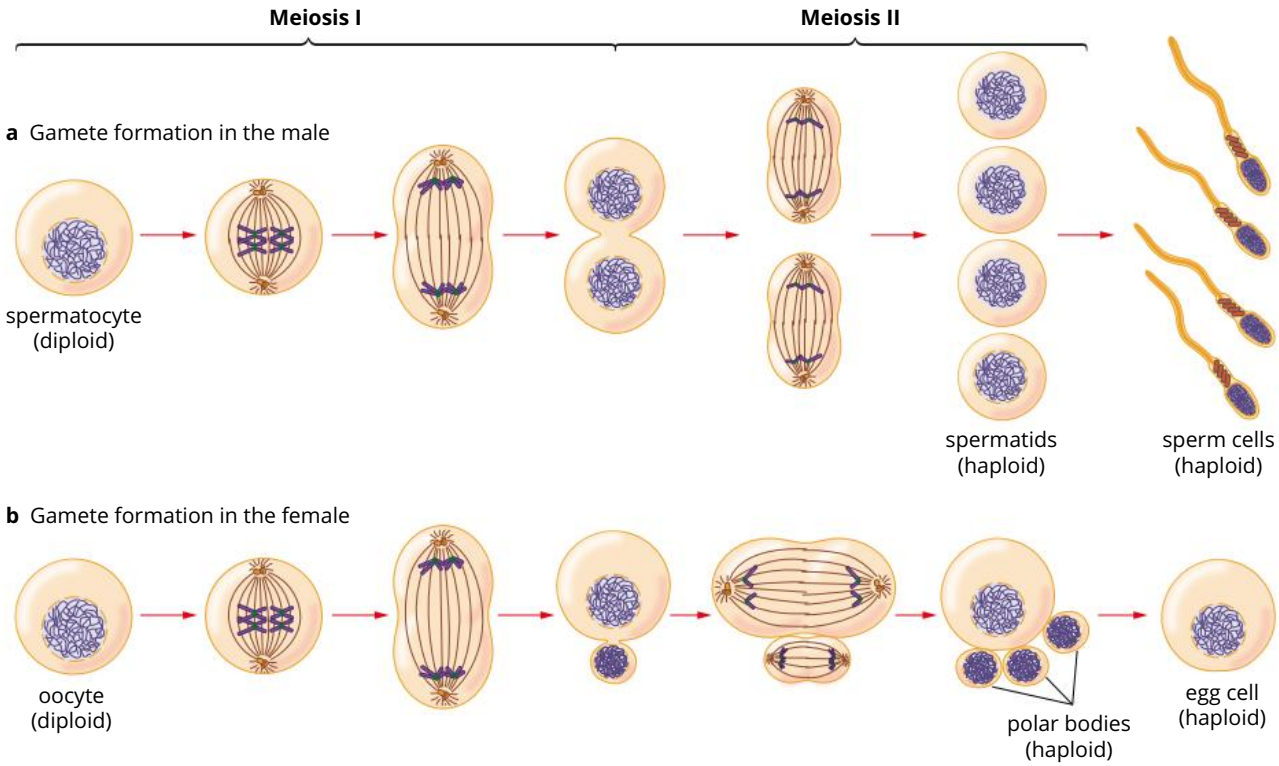


FIGURE 6.3.9 Haploid (a) male and (b) female gametes are produced by meiosis.

Ova (singular **ovum**), or eggs, are produced by a process called **oogenesis** (Figure 6.3.10). In humans, oogenesis begins before birth, but is arrested at two different stages during the life of the female. During embryonic development, germ cells in the developing ovaries undergo mitotic division to produce diploid **oocytes** (immature egg cells). Meiosis begins in all oocytes but is arrested at an early stage (prophase 1), producing primary oocytes.

A female is born with all her egg cells (primary oocytes) in her ovaries. Once puberty is reached and the ovarian cycle commences with menstruation, pituitary hormones cause continuation and completion of meiosis I, typically for a small number of oocytes from an ovary each ovarian cycle. This occurs under the influence of a follicle-stimulating hormone. The oocytes resume their meiotic division up to metaphase II and mature within a group of nutritive cells called a follicle. During this stage of the process, only one follicle with one oocyte will reach meiosis II, where the process is arrested in metaphase II. This is called a secondary oocyte, which is ovulated. Meiosis II only completes after fertilisation, producing another polar body. Only one egg forms from each oocyte during meiosis.

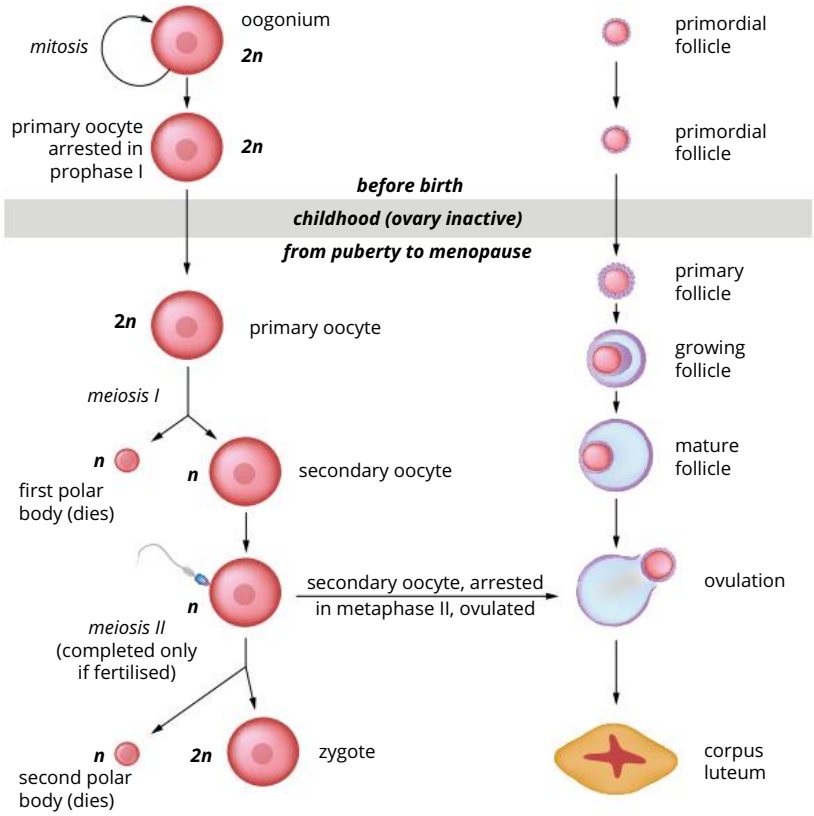


FIGURE 6.3.10 The process of oogenesis. Germ cells, called oogonia, divide mitotically to produce oocytes. Meiosis begins in the oocyte but is arrested at prophase 1. Once puberty is reached, meiosis resumes but is arrested again in metaphase II. Meiosis II only completes after fertilisation.

Comparing spermatogenesis and oogenesis

The similarities and differences between spermatogenesis and oogenesis are described in Table 6.3.2.

TABLE 6.3.2 Similarities and differences between spermatogenesis and oogenesis

Similarities	Differences
Both processes involve the formation of haploid gamete cells from an original diploid cell.	Spermatogenesis produces four haploid spermatozoa cells. Oogenesis produces only one haploid ovum: the other three haploid cells are much smaller and eventually degenerate.
Precursor cells undergo mitotic divisions before meiotic divisions occur.	The precursor cell in spermatogenesis is the spermatocyte. In oogenesis, it is the oocyte.
Hormones play a crucial role in initiating and controlling both processes.	In humans, spermatogenesis is a continuous process that begins at puberty, while oogenesis is a discontinuous process, tightly regulated beginning before birth and is only completed after fertilisation.

Spermatogenesis is a continuous process that produces four haploid gametes. Oogenesis is a discontinuous process that produces one haploid gamete.

6.3 Review

SUMMARY

- Gametes are the sex cells that combine in sexual reproduction.
- Sexual reproduction in multicellular organisms involves the fusion of gametes from two different individuals to form a zygote.
- Sexual reproduction involves equal genetic contributions from male and female parents.
- Meiosis is a division of the nucleus that halves the normal number of chromosomes and produces different genetic combinations in the haploid gametes.
- There are two sequential rounds of division in meiosis, called meiosis I and meiosis II. Meiosis proceeds through the following stages:
  - prophase I
  - metaphase I
  - anaphase I
  - telophase I and cytokinesis
  - prophase II
  - metaphase II
  - anaphase II
  - telophase II and cytokinesis.
- Homologous chromosomes are matching pairs of chromosomes in diploid organisms. These pairs of chromosomes carry the same genes, meaning that an individual inherits two copies of every gene.
- Chromatids of homologous chromosomes may exchange portions of their genetic information in a process called crossing over. The significance of crossing over is that it produces chromosomes with new combinations of genetic information. This is called recombination.
- Variation in gametes arises from the independent assortment of chromosomes and exchange of alleles through recombination during meiosis.
- Human sperm contributes a single set of 23 chromosomes to the new individual and determines its sex. The sperm cell contains 22 chromosomes and either an X or a Y chromosome.
- The human egg provides a single set of 23 chromosomes, including an X chromosome. It also provides nutrients for the growth of the embryo, and regulatory factors that control early development of the zygote.
- The process by which spermatozoa are produced from precursor cells by meiosis is called spermatogenesis. The ovum is produced by a process called oogenesis.
- Both oogenesis and spermatogenesis involve the formation of haploid gamete cells from diploid precursor cells through a process involving meiotic cell divisions.
- Spermatogenesis and oogenesis differ in many ways.
  - Spermatogenesis results in the production of four gametes, while oogenesis produces one gamete.
  - Spermatogenesis begins at puberty, while oogenesis begins during fetal development and is completed only after fertilisation.
  - Spermatogenesis is a continuous process, while oogenesis is discontinuous.

KEY QUESTIONS

Retrieval

1. Define haploid.
2. Identify the phase of meiosis in which crossing over occurs.
3. State the number of haploid daughter cells that result from meiosis.
4. Identify the process that results in each gamete receiving a random set of homologues.

Comprehension

5. Summarise the difference between a somatic cell and a gamete. Give an example of each.
6. Describe the main differences between mitosis and meiosis.
7. Explain how genetic variation is achieved during meiosis, referring to the phases of meiosis.
8. During the process of spermatogenesis, a precursor cell has the same amount of DNA as a typical somatic cell but half the number of chromosomes. Identify the cell and explain what phase of the cell cycle it is likely to be in.

Analysis

9. Compare the chromosomes of two daughter cells after meiosis I to the parent cell in the G1 phase.
10. Differentiate between prophase I and prophase II.
11. Differentiate between anaphase I and anaphase II.
12. Compare the processes of spermatogenesis and oogenesis.
13. Determine the chromosome number of the daughter cells, for a parent cell undergoing meiosis that has a chromosome number of 24 during interphase G1.

Chapter review

06

KEY TERMS

allosome
anaphase
autosome
binary fission
cell cycle
cell plate
cell replication
centriole
centromere
chiasma
chromatid
coenocyte
crossing over
cytokinesis
DNA helicase
DNA polymerase
DNA replication
fertilisation
gamete
germ cell
homologous chromosome
interphase
lagging strand
leading strand
meiosis
meiosis I
meiosis II
metaphase
mitosis
Okazaki fragment
oocyte
oogenesis
origin
ovum
prophase
recombination
replication fork
semi-conservative
sex chromosome
somatic cell
spermatocyte
spermatogenesis
spermatozoa
spindle
syncytium
telophase
zygote

KEY QUESTIONS

Retrieval

1 Define cell replication.

2 Identify the process that is not associated with cell division.
- **A** cytokinesis
- **B** DNA replication
- **C** pairing of homologous chromosomes
- **D** formation of two diploid daughter cells

3 Identify which of the following statements is true.
- **A** Cytokinesis is also called binary fission.
- **B** Cytokinesis involves the division of the nucleus.
- **C** Cytokinesis occurs during meiosis.
- **D** Cytokinesis occurs after mitosis.

4 Explain the role of DNA polymerase in the process of DNA replication.

5 Select the correct sequence of events for mitosis.
- **A** chromatids separate, chromosomes duplicate, cytokinesis occurs
- **B** cytokinesis occurs, chromosomes duplicate, chromosomes line up at the equator
- **C** chromosomes line up at the equator, chromatids separate, cytokinesis occurs
- **D** chromosomes duplicate, cytokinesis occurs, chromatids separate

6 Describe the cell plate.

7 Select the correct statement about chromosomes in mammalian gametes.
- **A** They are all identical to those in the parent cell.
- **B** They are different to those in the parent cell, but only because of mutation.
- **C** They are all identical to those in the parent cell, because crossing over and recombination between homologues does not create new combinations of alleles.
- **D** They are different to those in the parent cell, partly because of the effects of independent assortment.

8 Identify the correct reason that meiosis is a necessary process in living organisms.
- **A** It happens in the reproductive organs.
- **B** It is necessary for the growth of an organism.
- **C** It produces new cells to replace dead or dying cells.
- **D** It enables the production of gametes for sexual reproduction.

9 Select the phase of meiosis where crossing over occurs.
- **A** prophase I
- **B** anaphase I
- **C** metaphase II
- **D** anaphase II

10 State the name of the process that results in the production of spermatozoa.

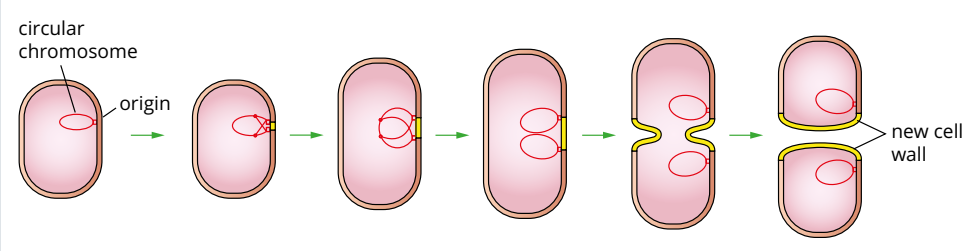
Comprehension

11 Describe the purposes of mitosis in:

a unicellular eukaryotes

b multicellular eukaryotes

12 Consider the following diagram:



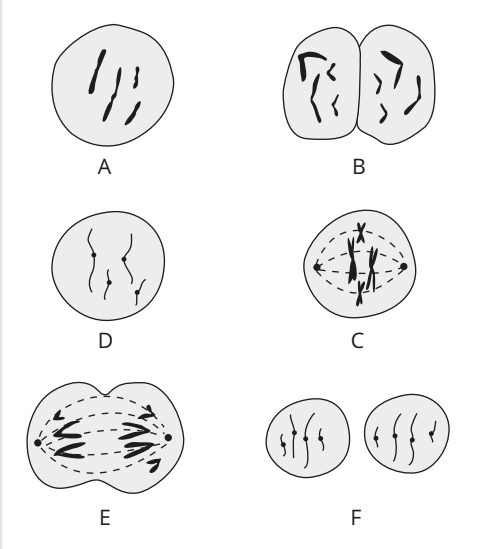
a Identify the process shown in the diagram.

b Determine one advantage and one consequence of this process when it is used as a form of reproduction.

13 Tabulate the different phases of the cell cycle, summarising what occurs during each phase.

14 Describe the three phases of DNA replication.

15 Determine the correct sequence of mitotic stages in the diagram below, and label the name of each stage.

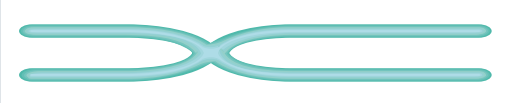


16 Describe the events that occur during interphase of the cell cycle.

17 Describe the process of oogenesis.

18 Explain how the movement of the replication fork results in the discontinuous formation of the lagging strand.

19 Examine the following diagram of a chromosome.



Deduce what phase of mitosis the chromosome first appears in this state.

20 The diploid number of chromosomes in horses is 64. Deduce how many chromosomes you would expect there to be in a horse's sperm cell.

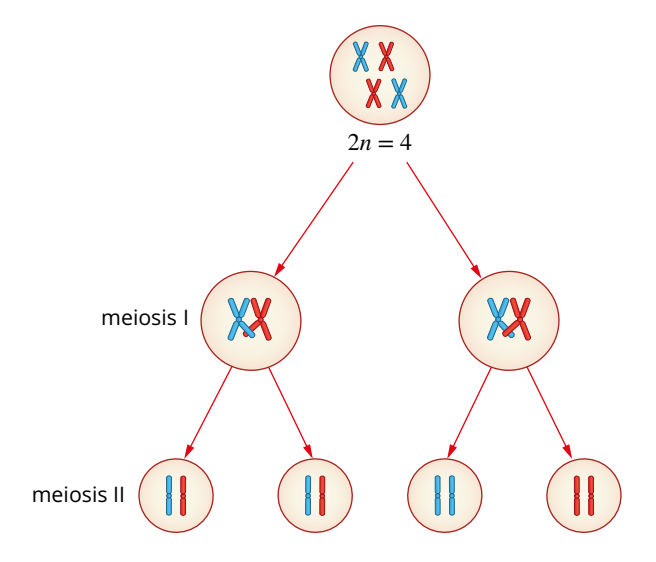
Analysis

21 Compare binary fission and mitosis.

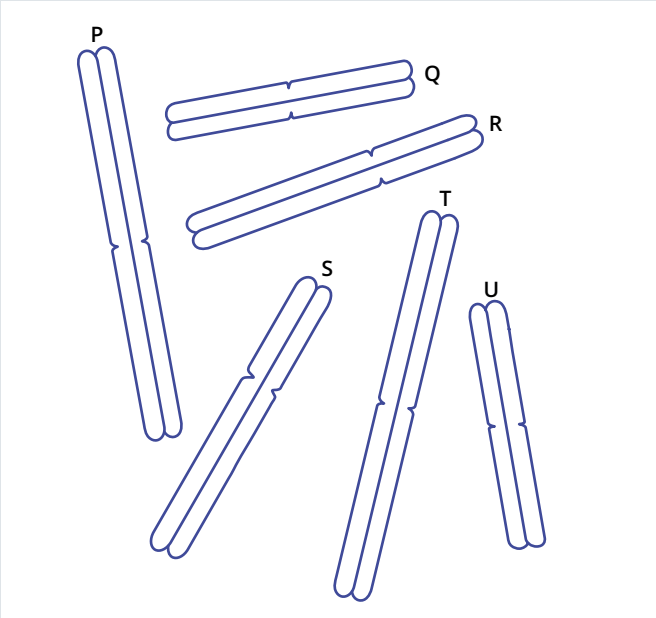
22 A student argued that all chromosomes consisted of two chromatids. Assess the student's understanding.

23 Contrast cytokinesis in plant and animal cells.

24 A student drew the following diagram to show her understanding of meiosis. Critique the diagram.



25 The figure below shows six chromosomes belonging to three homologous pairs.



Conclude, with justification, which chromosomes are homologous pairs.

26 Evaluate the statement: 'Offspring from the same parents show few variations in genotype'.

Knowledge utilisation

27 Anti-microtubule drugs causes the disassembly of microtubules and spindle. Predict what would happen if a cell were exposed to these drugs.

CHAPTER 07

Inheritance

Throughout history, people have wondered why children resemble their parents. Today we know that many characteristics are inherited, such as the colour of our hair, eyes and skin, as are many conditions such as haemophilia and colour-blindness. Sexual reproduction results in offspring with a set of unique characteristics inherited from their parents.

In this chapter, you will explore the complex interplay between DNA, genes, chromosomes and the traits they control. You will gain an understanding of how genes and their interactions determine the inheritance of characteristics from generation to generation. You will learn how to predict the inheritance patterns of traits using laws developed by Gregor Mendel, as well as learning about the role, structure and types of chromosomes. You will also discover how errors in chromosome replication lead to mutations that may have drastic impacts on the characteristics of an organism.

Syllabus subject matter

Topic 1 • DNA, genes and the continuity of life

MUTATIONS

- identify how mutations in genes* and chromosomes can result from errors in
  - DNA replication (point and frameshift mutation)
  - cell division (non-disjunction)
  - damage by mutagens (physical, including UV radiation, ionising radiation and heat and chemical)
- explain how non-disjunction leads to aneuploidy
- use a human karyotype to identify ploidy changes and predict a genetic disorder from given data
- describe how inherited mutations can alter the variations in the genotype of offspring.

INHERITANCE

- predict frequencies of genotypes and phenotypes using data from probability models (including frequency histograms and Punnett squares) and by taking into consideration patterns of inheritance for the following types of alleles: autosomal dominant, sex-linked and multiple
- define polygenic inheritance and predict frequencies of genotypes and phenotypes for using three of the possible alleles.

* The greyed out sections of this dot point are addressed specifically in Chapter 5.

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7.1 Chromosomal abnormality

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand that different species have different numbers of chromosomes
- differentiate between allosomes and autosomes
- recall the human karyotype and recognise that errors in the karyotype result in genetic abnormalities
- explain non-disjunction and aneuploidy.

Chromosomes are the structures that allow DNA to be maintained in discrete packages. Their structure protects the integrity of the information stored on DNA and reduces the chance of error in replication. All organisms have their genetic material stored in some form of chromosome. An understanding of the structure and function of chromosomes is essential for understanding inheritance. Chromsomes are discussed in detail in Module 5.1.

KARYOTYPES

When examining chromosomes and identifying pairs, look at:

- chromosome length
- centromere position
- banding patterns.

The coiling and supercoiling of DNA found in eukaryotic nuclei produces chromosomes (Module 5.1). Scientists examine eukaryotic chromosomes when they are most visible in the cell—at the metaphase stage of the cell cycle. The chromosomes are stained so that characteristic patterns of light and dark bands (G bands) appear along the arms of the chromosomes. The bands reflect regional differences in the amounts of bases A and T versus G and C.

Homologous chromosomes have the same gene loci, so chromosomal length and the banding patterns of homologous chromosomes are similar. In other words, banding patterns are specific and consistent. They can be used to distinguish between chromosomes and to identify subtle changes in chromosome structure that may be associated with genetic abnormalities (Figure 7.1.1). Specific banding patterns can also be used to identify the same chromosomes or regions of chromosomes across different species.

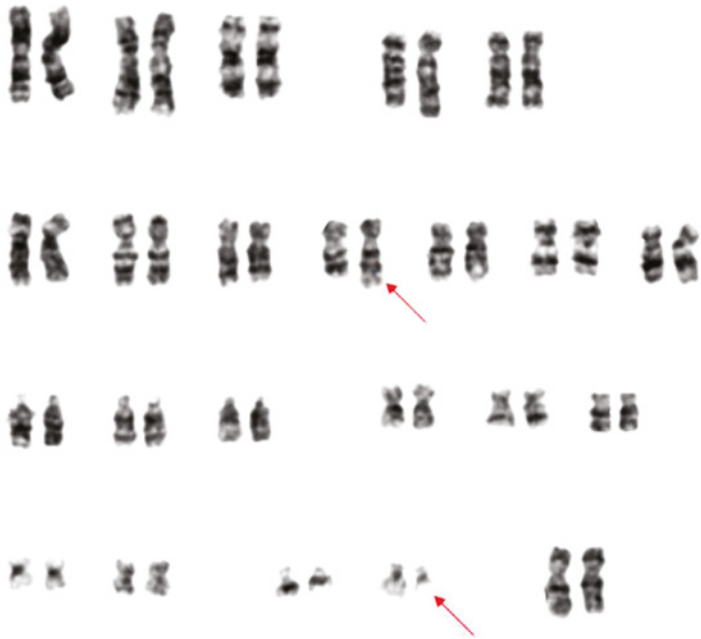


FIGURE 7.1.1 The banding pattern on the chromosomes indicated with the arrow shows researchers where mutations have occurred.

A **karyotype** is the image or picture of the full set of chromosomes from an individual's cell (Figure 7.1.2). A karyotype is represented by photographs or diagrams of the homologous chromosomes arranged in pairs according to their length and the position of the centromere. Karyotypes allow scientists to compare the chromosome sets of related species. Karyotypes also allow scientists to identify changes that may be associated with genetic abnormalities, such as:

- changes in chromosome number (the loss or gain of whole chromosomes)
- changes in structure (such as the duplication, inversion or deletion of part of a chromosome).

The human karyotype

In humans (and some other organisms), sex chromosomes are distinguished from the remaining chromosomes (after the homologous autosomes have been paired). A karyotype for a human male shows that there are 22 pairs of autosomes and two sex chromosomes, X and Y. Sex chromosomes are also referred to as allosomes (Module 5.1). A karyotype for a human female shows 22 pairs of autosomes and two X chromosomes (Figure 7.1.2). The autosomal pairs are numbered 1 to 22 and ordered from largest to smallest. The allosomes are usually shown after the autosomes.

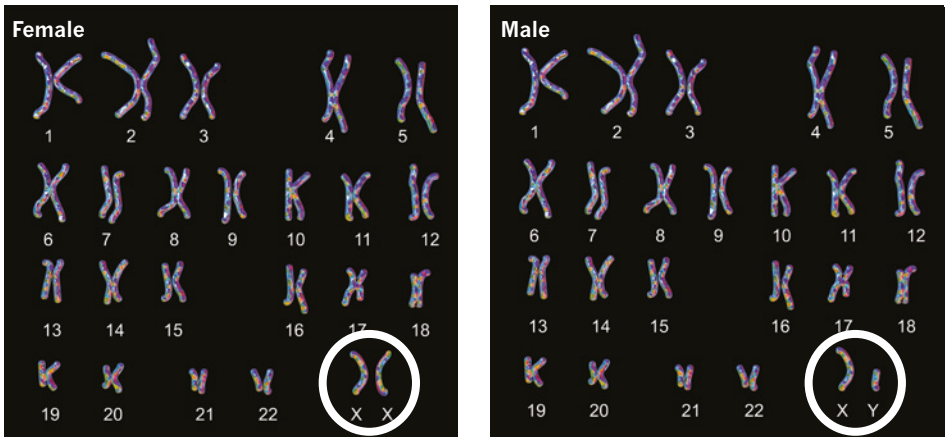


FIGURE 7.1.2 The human karyotype with the sex-determining chromosomes circled.

CHROMOSOMAL ABNORMALITIES

The expected normal karyotype is not always observed in individuals. This is because chromosomal mutations and other abnormalities may occur. The two major forms of chromosomal abnormalities are block mutations and aneuploidy.

Block mutations

As shown in Module 5.4, genetic mutations can occur within an individual gene as a result of altering a single nucleotide or small sequence of nucleotides. However, mutations can also affect genetic material at a chromosomal level. Mutations that affect large sections of a chromosome, typically multiple genes, are called **block mutations** (or chromosomal mutations). These types of mutations usually occur during meiosis in eukaryotic cells. They can also be caused by mutagens such as radiation. When a gene is disrupted by the mutation, the effects are serious, even lethal. There are five main forms of block mutations:

- duplication
- deletion
- inversion
- insertion
- translocation.

Duplication mutations

Duplication mutations involve the replication of a section of a chromosome that results in multiple copies of the same genes on that chromosome (Figure 7.1.3). There can be thousands of repeats and the repeats can increase gene expression.

One form of a common inherited neurological disorder called Charcot-Marie Tooth (CMT) disease results from the duplication of the gene on chromosome 17. This gene codes for a protein in peripheral nerve myelin. As a result of this duplication mutation, the expression of protein-22 in the peripheral nerve cells is affected, which causes a slow degeneration of the peripheral nerves in the feet, legs, arms and hands. The disease is not life-threatening. Approximately one in 2500 people in Australia have been diagnosed with CMT disease.

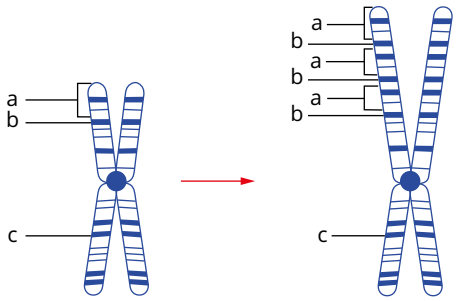


FIGURE 7.1.3 Chromosomal duplication mutations result in multiple repetitions of a sequence of DNA.

Deletion mutations

Deletion mutations remove sections of a chromosome (Figure 7.1.4). Deletions lead to disrupted or missing genes and the resultant change in gene expression can have serious effects on growth and development. Chromosomal deletions are often fatal. Wolf-Hirschorn syndrome is an example of the deletion of the short arm of chromosome 4. Symptoms include epilepsy, facial deformities and delayed bone development. People with this syndrome who survive infancy have severe disability and immune deficiency.

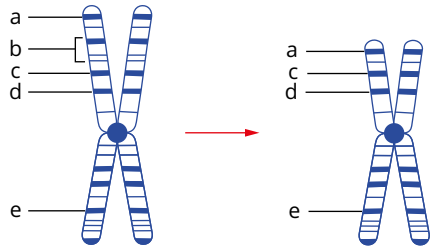


FIGURE 7.1.4 Chromosomal deletion mutations involve the loss of large sequences of DNA from the chromosome (sometimes whole genes).

Inversion mutations

During an **inversion mutation**, a section of the double-stranded polynucleotide chains break off the chromosomes, rotates 180° and reattaches to the same chromosome (Figure 7.1.5). Inversions may involve as few as two bases or they may involve several genes. Haemophilia A is one example of an inversion mutation. The mutation occurs in the factor VIII gene on the X chromosome. This particular type of block mutation is found in approximately 43% of haemophilia A patients.

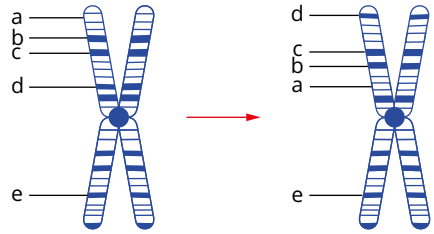


FIGURE 7.1.5 Chromosomal inversion mutations involve a broken section of the sequence rotating 180° before reattaching.

Insertion mutations

An **insertion mutation** occurs when a section of one chromosome breaks off and attaches to a different chromosome (Figure 7.1.6). In eukaryotes, the effects of this type of mutation depend on whether the cell retains two copies of every gene. During meiosis, if the chromosome now containing the insertion is separated from the chromosome in which the material originated, some gametes may have two copies of the genes in the inserted section, while others will be missing them entirely (Figure 7.1.7).

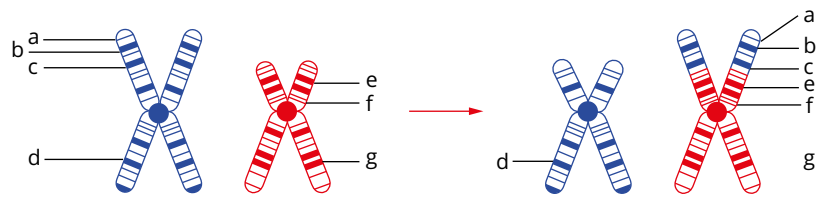


FIGURE 7.1.6 Chromosomal insertion mutations involve a sequence breaking off one chromosome and attaching to another.

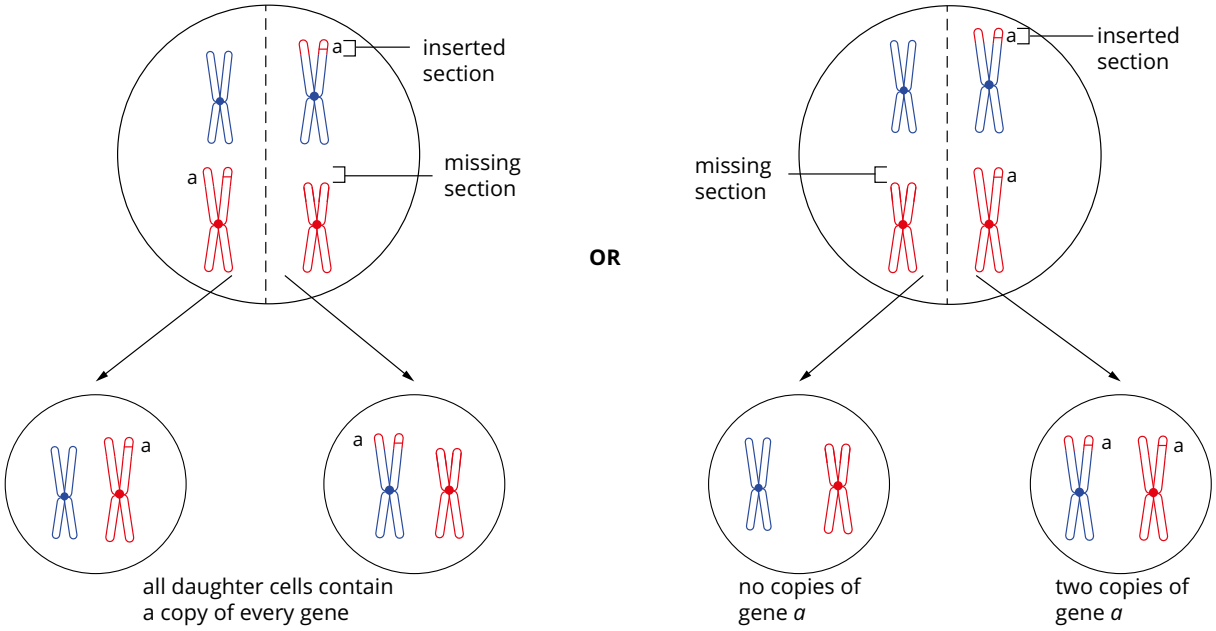


FIGURE 7.1.7 As a result of random assortment in meiosis, a chromosomal insertion mutation may lead to daughter cells with one, two or no copies of the inserted region. This image depicts meiosis I only.

Translocation mutations

In **translocation mutations**, a whole chromosome or a segment of a chromosome becomes attached to or exchanged with another chromosome or segment. For example, sections from two non-homologous chromosomes may break off at the same time. They may reattach to the other chromosome, swapping genetic material (Figure 7.1.8). Translocations typically interrupt normal gene regulation and are the cause of some forms of cancer, such as leukaemia and Ewing's sarcoma.

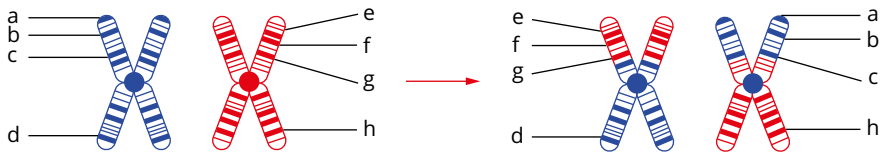


FIGURE 7.1.8 Chromosomal translocation mutations can involve two different chromosomes exchanging segments.

Non-disjunction and aneuploidy

Under certain circumstances during meiosis and mitosis, chromosomes fail to separate correctly. This is referred to as **non-disjunction**. When there is an error in chromosome separation, the result is an abnormal number of chromosomes in the daughter cells. This abnormal number of chromosomes is referred to as **aneuploidy**.

During mitosis, non-disjunction occurs when sister chromatids fail to separate correctly. During meiosis, non-disjunction is the result of homologous chromosomes incorrectly separating. Figure 7.1.9 (page 330) shows non-disjunction in meiosis I and meiosis II.

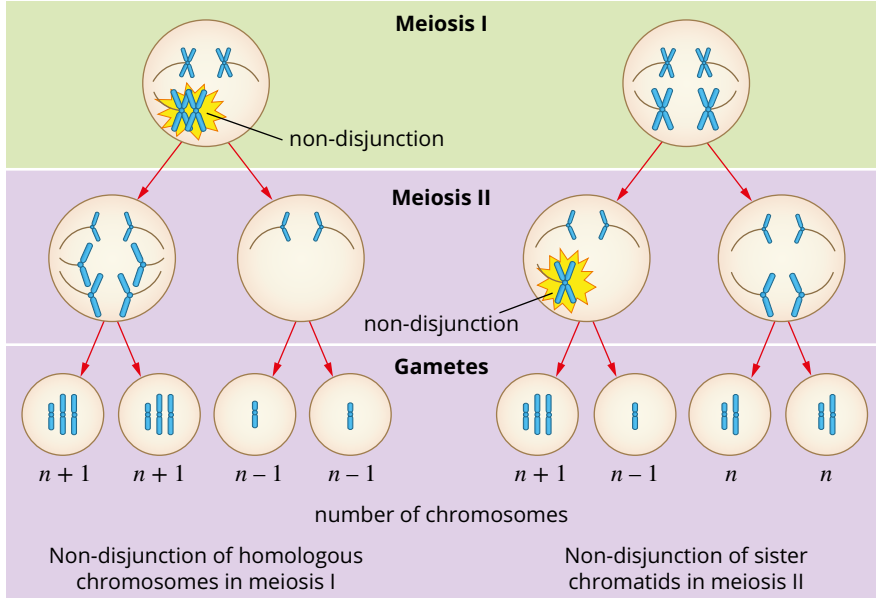


FIGURE 7.1.9 Non-disjunction occurring in meiosis.

Using karyotypes to identify chromosomal number abnormalities

A number of syndromes result from aneuploidy. Down syndrome is a typical example of a syndrome that results from having one extra chromosome. It results from one extra copy of chromosome 21, which can be identified via a karyotype (Figure 7.1.10). This type of abnormality is called a trisomy, because there are three copies of the chromosome.

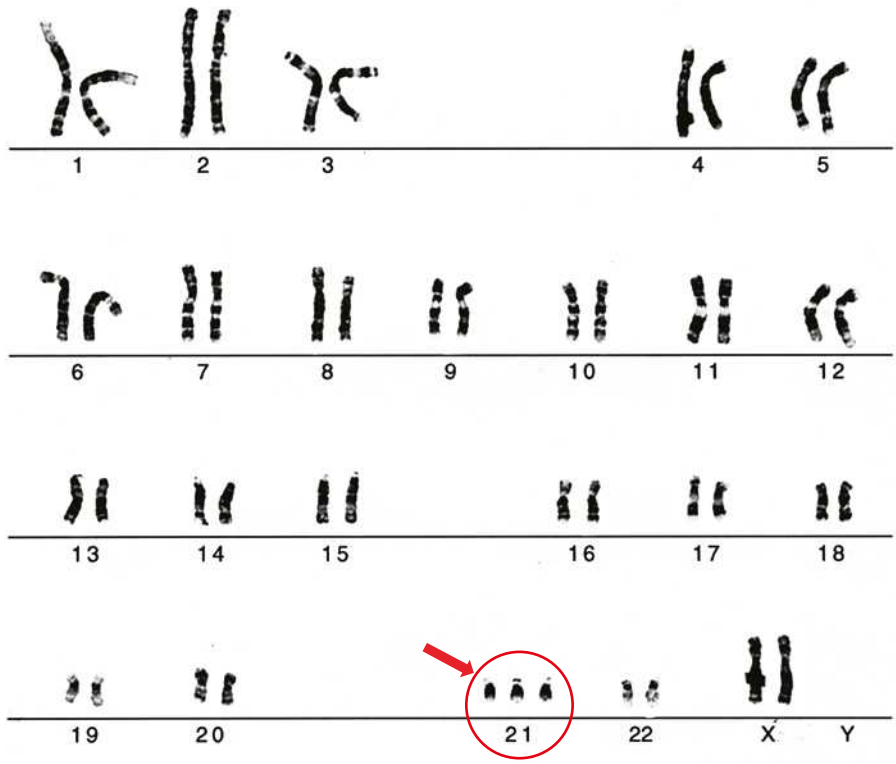


FIGURE 7.1.10 The Down syndrome karyotype, showing an extra copy of chromosome 21.

Two other abnormalities formed as a result of an abnormal chromosome number are Klinefelter syndrome and Turner syndrome. In Klinefelter syndrome (also called XXY syndrome), males have two X chromosomes and one Y chromosome instead of one X and one Y chromosome (Figure 7.1.11). As a result, they have 47 chromosomes. Males with Klinefelter syndrome are infertile and have other characteristics such as breast development and a tall stature.

In Turner syndrome (also called monosomy X), which occurs only in females, there is one X chromosome instead of two (Figure 7.1.12). People with Turner syndrome are infertile and have a short stature.

Table 7.1.1 shows some of the consequences in humans of abnormal chromosome numbers. This is the result of abnormal meiosis in one of the parents of the person with the condition.

TABLE 7.1.1 Conditions in humans that are a result of errors during meiosis and genetic recombination

Condition	Chromosome change	Traits of person with condition
Down syndrome	• three copies of chromosome 21 present (trisomy 21) • 47 chromosomes	• male or female • some intellectual disability • characteristic palm prints and facial features • may be infertile
Klinefelter syndrome	• extra X chromosome (XXY) • 47 chromosomes	• male • sterile • often some intellectual disability • female secondary sex traits (e.g. breast enlargement)
Patau syndrome	• three copies of chromosome 13 present (trisomy 13) • 47 chromosomes	• male or female • small skull • intellectual disability • cleft lip • cleft palate • usually has heart defects • seldom survives more than four months after birth
Turner syndrome	• all or part of one X chromosome is altered or missing (monosomy) • 45 complete chromosomes	• female • short stature • infertile • fluid retention and puffiness in hands and feet • kidney and heart problems • some learning difficulties but usually has normal intelligence

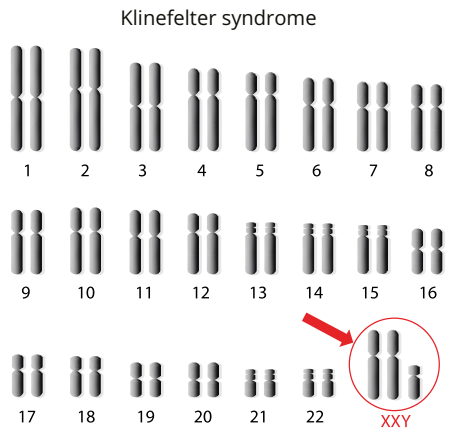


FIGURE 7.1.11 Klinefelter syndrome karyotype, showing an extra copy of the X chromosome.

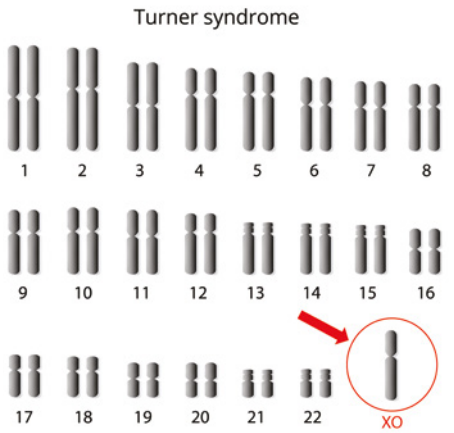


FIGURE 7.1.12 Turner syndrome karyotype, showing only one copy of the X chromosome.

WS 4.1.8

7.1 Review

SUMMARY

- Chromosomes vary in size, banding pattern and centromere position.
- Homologous chromosomes are matching pairs of chromosomes (one set from each parent) that have the same genes.
- Sex chromosomes (allosomes) are chromosomes that are involved in sex determination.
- In humans the sex chromosomes are X and Y.
- Females have two X chromosomes and males have an X and Y.
- Other organisms may have other sex chromosomes, or not at all.
- Autosomes are chromosomes that are not involved in sex determination.
- A karyotype is the number and appearance of a cell's chromosomes.
- A karyotype can identify:
  - the number of chromosomes in a cell
  - the gender of the individual
  - whether an individual has an extra chromosome, such as in Down syndrome and Klinefelter syndrome
  - where an individual is missing a chromosome, such as in Turner syndrome
  - the position of the centromere
  - the size of the chromosome.

KEY QUESTIONS

Retrieval

1 Recall how many homologous pairs of chromosomes you would expect to find in the cells of most:
- **a** human females
- **b** human males

2 Explain aneuploidy.

3 State the chromosomal variations for the aneuploidy syndromes listed below.
- **a** Down syndrome
- **b** Turner syndrome
- **c** Klinefelter syndrome

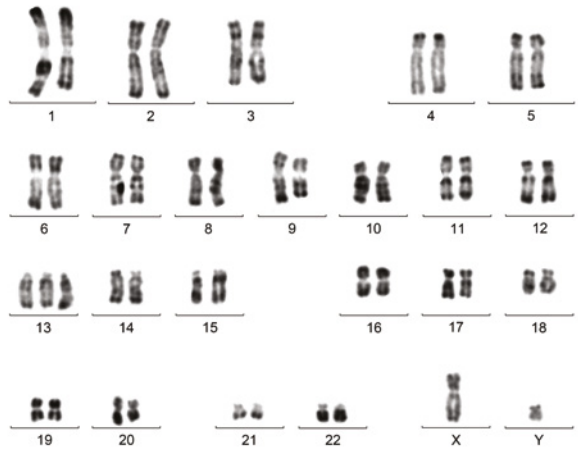
Comprehension

4 Describe how karyotypes can be used by scientists to determine information about chromosomes.

5 Explain the effect duplication mutations have on chromosomes.

Analysis

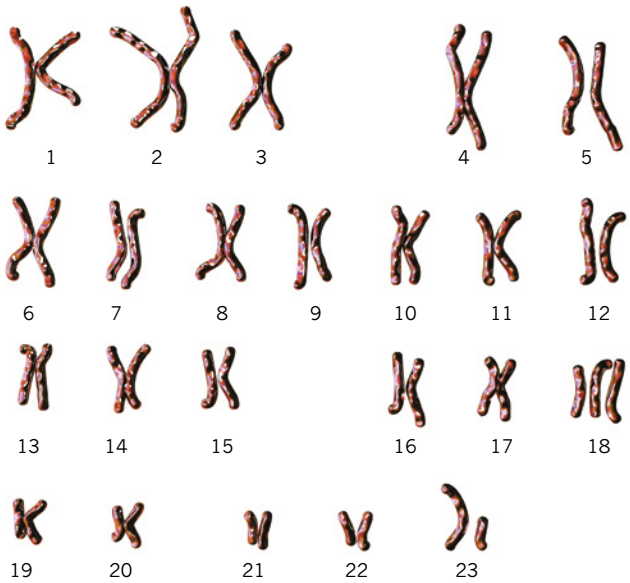
6 The figure below shows a human karyotype.
- **a** Determine the sex of the individual.
- **b** Justify whether or not this is a normal karyotype.



7 Using your knowledge of the human karyotype, examine the image below.

a Determine if this individual has trisomy 21.

b Explain how you made your diagnosis and comment on whether any other diagnoses can be made.



8 The image below shows the homologous pair of chromosome 5 in a person who suffers from cri du chat syndrome. Identify the abnormal chromosome and determine the chromosomal disorder.

9 The karyotype below is from a miscarried fetus.

a Determine the sex of the fetus.

b Identify the chromosomal disorder.

c Explain how the disorder may have occurred.

7.2 Genotypes and phenotypes

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recall the difference between genotype and phenotype
- understand how genes and alleles are named and written
- explain dominance, recessiveness, co-dominance and incomplete dominance
- understand and interpret frequency histograms.

The names of genes and alleles are always italicised.

Since the work of Gregor Mendel, the fundamentals of gene expression have been crucial to the understanding of inheritance. This module discusses the key principles of genotype and phenotype, along with monogenic and polygenic traits.

GENOTYPE

A **genotype** is the set of alleles present in the DNA of an individual organism. It is the result of inheritance. In Chapters 5 and 6, you learned that an allele is an alternative form of a gene. Each individual usually only has two alleles for each gene: one inherited from their mother and one inherited from their father. However, one gene may have many alleles, and this is what leads to variation in a population. Characteristics that are determined by a single gene are called **monogenic** traits.

Multiple alleles at a locus

Consider a gene, which we will call gene *A*, which has two alleles. Different alleles are represented by different symbols. One allele can be represented by an upper-case *A*, and the other by a lower-case *a*.

If the locus of gene *A* is on a homologous chromosome, there will be two copies of the gene in a diploid cell (the maternal and paternal copy). If you inherited the allele *A* from both parents, your genotype for gene *A* will be *AA*. If you inherited the allele *A* from one parent and the allele *a* from the other parent, you will have the genotype *Aa*. If you inherited the allele *a* from both parents, you will have the genotype *aa*.

Therefore, there are three different combinations or genotypes of gene *A*: *AA*, *Aa* or *aa* (Figure 7.2.1). Genotypes *AA* and *aa* contain only one type of allele, so the individual is said to be homozygous for that gene and is called a **homozygote**. Genotype *Aa* contains two different alleles, so the individual is said to be heterozygous for that gene and is called a **heterozygote**.

Now consider a gene, which we will call gene *B*, on the X chromosome. Because this gene is on the X chromosome, it is said to be **X-linked**. Gene *B* also has two alleles, *B* and *b*. To show that this gene is on the X chromosome and therefore X-linked, the symbol 'X' is used, with a superscript to represent the gene's allele. So the two alleles are named X^B and X^b (Figure 7.2.2).

Because females have two copies of the X chromosome, they can be homozygous (X^BX^B or X^bX^b) or heterozygous (X^BX^b) for gene *B*.

Males only have one copy of the X chromosome, so they are referred to as being **hemizygous** ('hemi' means 'half'). This term indicates that a male has only half the number of copies of genes on the X chromosome compared with a female. The Y chromosome is used in describing the genotype to emphasise that the individual is a male. As shown in Figure 7.2.2, the two possible genotypes of gene *B* for a male are X^BY and X^bY.

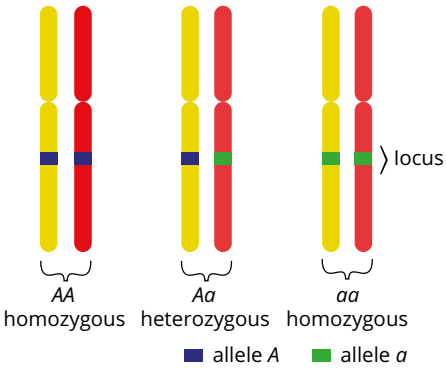


FIGURE 7.2.1 On homologous chromosomes, alleles of a gene occur at the same locus. If a gene has two alleles, there can be three different combinations or genotypes. Two of these combinations are homozygous, and one is heterozygous.

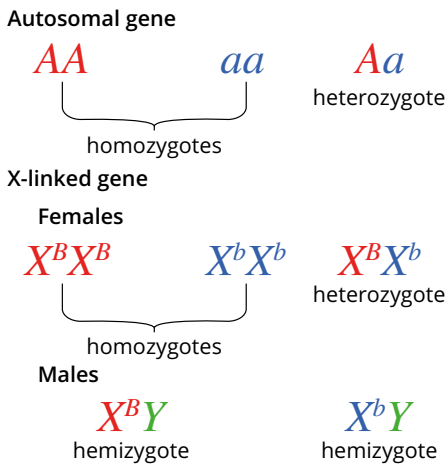


FIGURE 7.2.2 The symbols used to record genotypes of autosomal and X-linked genes.

Naming genes

There are internationally accepted names for genes and their abbreviated forms. For example, the gene that codes for phenylalanine hydroxylase, an enzyme involved in the inherited disorder phenylketonuria, is abbreviated to *PAH*. The gene name is always italicised, to distinguish genes from the proteins they encode. For example, BRCA1 is an enzyme expressed in the cells of breast and other tissue, where it helps repair damaged DNA or destroy cells if DNA cannot be repaired. The gene that codes for this enzyme is known as *BRCA1* (breast cancer gene 1).

PHENOTYPE

An organism's **phenotype** is all of its observable characteristics. It is the result of inheritance such as genotype but also the effects of the organism's environment. When studying inheritance, it is important to know the genotypes of parents and offspring. However, it is equally important to know the specific observable characteristics, or phenotype, that can result from a given genotype. The phenotype includes any distinct property of an organism: physical, chemical, physiological or behavioural. In experimental crosses (matings), such as those carried out by Gregor Mendel, phenotypes are observed to determine the underlying genotypes.

Influences on phenotype

An example of a phenotype is skin colour. Your skin colour depends on how much skin pigment (melanin) you produce, which is determined by your genotype. Your skin colour phenotype also depends on environmental factors such as exposure to sunlight, especially in pale-skinned people, which stimulates the activity of the genotype. The greater the exposure, the more melanin is produced temporarily until the exposure is ceased (Figure 7.2.3).

FIGURE 7.2.3 The effect of sun exposure on skin colour. The darker part of the skin has been exposed and has produced more melanin, causing it to darken (neck and shoulders). Unexposed skin (back) does not produce extra melanin.

The amount of melanin that is able to be produced in response to environmental factors is dependent on inherited regulatory genetics and the genotype. The genotype determines the possible range of phenotypes for a particular characteristic or trait, and the environment influences where in that range the actual phenotype will be.

In Arctic foxes, two fur colour genotypes occur, called 'white morph' and 'blue morph'. The fur of the blue morph remains dark blue-grey throughout the year, but the fur of the white morph varies from dark brown or grey to pure white. In summer the fur is dark, but as winter approaches the fur gradually changes to white in response to the increasing cold and shorter day length (Figure 7.2.4). At the end of winter the fur gradually returns to its summer colour.

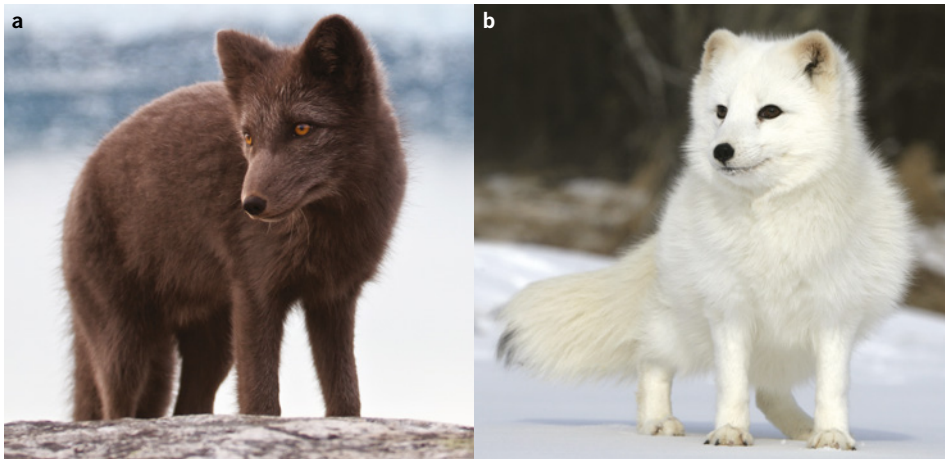


FIGURE 7.2.4 The fur of the white morph genotype of the Arctic fox changes from (a) dark brown or grey to (b) pure white.

Phenylketonuria

The inherited disorder phenylketonuria (PKU) is a consequence of the build-up of an amino acid called phenylalanine in the blood. This is toxic to developing neurons, leading to abnormal development of the nervous system and intellectual disability. PKU is caused by a mutation in the *PAH* gene, which codes for an enzyme that converts phenylalanine into another amino acid, tyrosine. If an individual inherits two copies of the mutant allele (that is, they are homozygous for the gene), they will develop PKU. Fortunately, development of the symptoms can be prevented by modifying the diet (environment) of babies that test positive for PKU shortly after birth. If homozygous individuals reduce their intake of dietary phenylalanine, particularly during childhood, they show normal brain development. Newborns in Australia are routinely tested for genetic disorders like PKU.

Fur colour in Himalayan rabbits

The coat colour of Himalayan rabbits provides an example of the effect of environmental temperature on phenotype. The Himalayan rabbit is homozygous for a mutant allele that encodes for a heat-sensitive enzyme called tyrosinase. Tyrosinase catalyses the production of melanin and other pigments. At normal body temperature, tyrosinase is produced but it is inactivated, resulting in no melanin being produced and a white coat. At low temperatures, tyrosinase is activated and results in the formation of melanin, causing black fur to form. When a small section of fur is shaved from a white region on the back, the fur grows back black if the animal is kept at low temperatures, but white if the animal is kept at high temperatures (Figure 7.2.5).

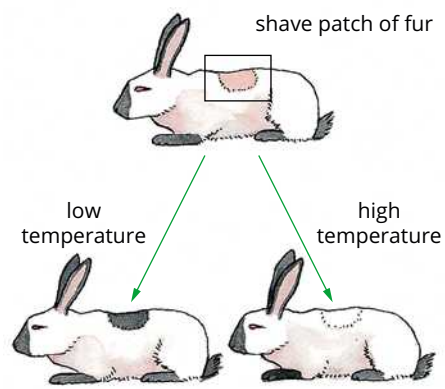


FIGURE 7.2.5 The relationship between temperature and fur colour in Himalayan rabbits.

Flower colour in hydrangeas

Hydrangeas are a commonly seen example of environmental effects on phenotype. If cuttings of a single hydrangea plant are grown in very acidic soil (pH 5.5 or less), the flowers produced are blue. If the cuttings are grown in weakly acidic or alkaline soil (pH 6.5 or higher), the flowers are pink (Figure 7.2.6). The cuttings are of identical genotype, so it must be the environment (the pH of the soil) that affects the phenotype of the hydrangea.

FIGURE 7.2.6 Flower colours of cuttings of the same hydrangea plant grown in an acid soil (left) and an alkaline soil (right).

This effect is caused by the relationship between soil pH, a pigment called anthocyanin and the availability of aluminium in the soil for uptake by the plant. At a soil pH of 5.5 or less, aluminium is free to be taken into the plant. Anthocyanin is normally red, but it binds to aluminium in the plant to form a blue pigment called metalloanthocyanin, resulting in blue flowers. At a soil pH of 6 or higher the aluminium binds to soil particles and is less available to the plants. This leaves most of the anthocyanin in the plant in its red form, resulting in pink flowers.

This illustrates that genotypes establish the coded information for traits, and then the environment influences the expression of the alleles in the genotype to physiologically respond to the environment. Some alleles have a wide range of responses, meaning the amount of expression of the gene can vary significantly or alternative splicing can result in numerous variations of the proteins produced (see Chapter 5 and 6), hence the results in varied phenotypes. Other alleles have a limited range in the amount that the gene is expressed and there are few options for alternative splicing.

GENETIC DOMINANCE

The relationship between genotype and phenotype gives an insight into an important property of phenotypes known as **dominance**. For a given gene, the phenotype of the heterozygote compared with the appearances of each homozygote allows us to determine whether a phenotype is completely dominant, co-dominant or recessive. It is important to understand that dominance and recessiveness are properties of alleles, not genes. They are expressed as dominant or recessive phenotypes. Genes are neither dominant nor recessive.

Complete dominance

To understand complete dominance it is useful to consider the white eye gene in blowflies. There are two alleles for the white eye gene, *W* and *w*. Individuals with genotype *WW* have red eyes, while individuals with genotype *ww* have white eyes (Figure 7.2.7). Individuals with genotype *Ww* do not show an in-between trait such as pink eyes, but instead have red eyes, making them indistinguishable from those of the *WW* genotype.

Blowflies with genotype *Ww* display the red eye because the *W* allele makes enough membrane transporter protein to give the eye normal red pigment levels. The red eye colour phenotype is referred to as the **dominant phenotype**, because it only needs one *W* allele for that phenotype to be displayed. The white eye colour phenotype is referred to as the **recessive phenotype** because it is not observed in the heterozygote. It needs two copies of the *w* allele for it to be observed in the phenotype. This example shows that scientists can determine if a phenotype is dominant only by examining the heterozygote.

By convention, the allele associated with a dominant phenotype is represented by an upper-case symbol (e.g. *W*). The allele associated with a recessive phenotype is represented by a lower-case symbol (e.g. *w*). The blowfly's white eye is an example of complete dominance. *Ww* individuals have the same eye colour as *WW* flies.

FIGURE 7.2.7 Blowflies with the *WW* and *Ww* genotypes have a red-eye phenotype (top). Blowflies with the *ww* genotype have a white-eye phenotype (bottom).

Co-dominance

In **co-dominance** the full phenotypic expression of both alleles is observed. The classic example is the ABO human blood group system. Figure 7.2.8 shows red blood cells displaying the four possible phenotypes: AB, A, B and O. In the ABO blood group system, there are three allelic forms (I^A, I^B and i) at the same locus and individuals can have A, B, AB or O phenotypes. Those with the less-common AB blood group are heterozygotes carrying one allele (I^A) that produces the A antigen (a polypeptide) and one allele (I^B) that produces a B antigen (a different polypeptide).

Because both the A and B antigens are present on the surface of red blood cells and can be detected using appropriate antibodies, neither phenotype is truly dominant. This is co-dominance. Thus, A and B phenotypes are co-dominant and the O phenotype is recessive.

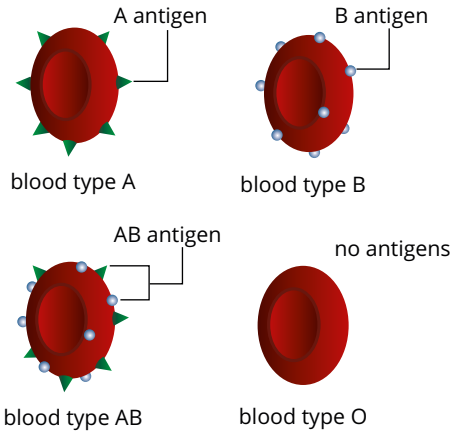


FIGURE 7.2.8 The four possible phenotypes of red blood cells: AB, A, B and O.

Incomplete dominance

Sometimes neither phenotype is completely dominant, and intermediate phenotypes occur. The presence of intermediate phenotypes occurs when one allele for a specific trait is not completely expressed over another allele. This form of dominance is known as **incomplete dominance**.

An example of incomplete dominance is found in snapdragons (Figure 7.2.9). Homozygous snapdragon flowers can have a red flower phenotype (R_1R_1) or a white flower phenotype (R_2R_2). In this case upper-case letters and subscripts are used to distinguish the alleles because neither phenotype is completely dominant. Plants of the R_1R_2 genotype have pink flowers. In R_1R_1 flowers, both copies of the R_1 allele produce an enzyme required to produce red pigment. In R_2R_2 flowers, no pigment is produced because the R_2 allele produces either no enzyme or a defective enzyme.

Since the R_1R_2 flower has one R_1 allele, which produces the active enzyme, and one R_2 allele, which does not, it will produce half the amount of pigment as the R_1R_1 flower. The resulting flower is pink.

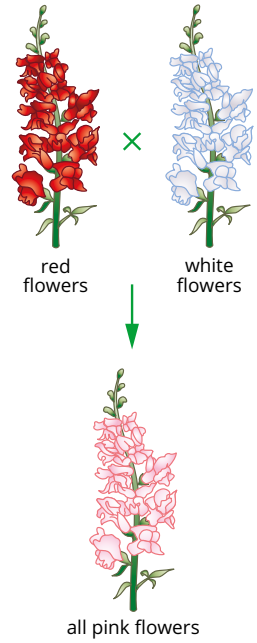


FIGURE 7.2.9 When red (R_1R_1) (left) and white (R_2R_2) (centre) snapdragons are crossed, the resulting heterozygotes are pink (R_1R_2) (right) because only half the amount of red pigment is produced.

MONOGENIC AND POLYGENIC TRAITS

An individual's phenotype is dependent on the alleles present and, depending on the trait, environmental conditions. Some phenotypes are determined by one set of genes. These phenotypes are considered to be monogenic. Phenotypes that are determined by the interaction of multiple genes are said to be **polygenic**.

Monogenic traits

Many traits, such as seed colour and texture in the case of Mendel's pea experiments, are controlled by the expression of single genes, hence the term monogenic. Monogenic traits tend to have very predictable inheritance patterns and a smaller range of phenotypes than polygenic traits.

The human ABO blood group is an example of a monogenic trait. As discussed above, the human ABO blood group system is determined by three alleles, represented as I^A, I^B and i. Allele I^A produces the A antigen, I^B produces the B antigen, and i produces no antigen. Each person carries two copies of these three possible alleles (a maternal and paternal copy). There are therefore six possible genotypes and four phenotypes (Figure 7.2.10).

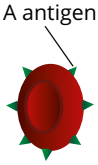
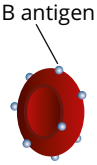
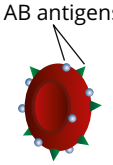
ABO blood groups				
Blood type	**Type A**	**Type B**	**Type AB**	**Type O**
Possible allele combinations	$I^A I^A$ $I^A i$	$I^B I^B$ $I^B i$	$I^A I^B$	$i\ i$
Antigen (on RBC)	A antigen 	B antigen 	AB antigens 	no antigens

FIGURE 7.2.10 The ABO blood group system is based on three alleles. The production of antigens A and B depends on the combination of alleles present.

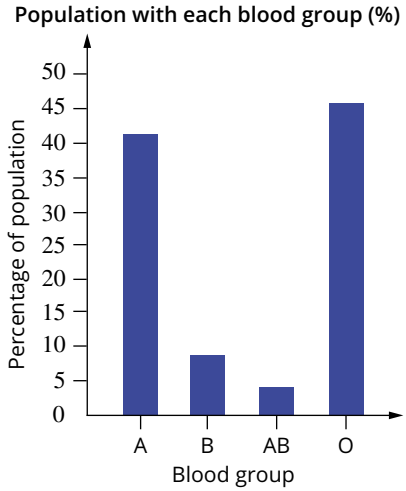


FIGURE 7.2.11 The ABO blood system is an example of discrete variation.

When only one gene with a few alleles controls a trait, and the environment has little effect on the phenotype, **discrete variation** of the phenotypes is observed. Discrete variation (also known as discontinuous variation) is when observed individuals show distinct classes or categories. Discrete variation of phenotypes is a characteristic of monogenic traits. The ABO blood system, described above, is one example of discrete variation of a monogenic trait (Figure 7.2.11).

Polygenic traits

For some traits, such as skin colour and height in humans, more than one gene (and a number of alleles for each gene) contributes to the phenotype of an individual. This is known as **polygenic inheritance** and results in a much greater range of phenotypes. Polygenic traits in non-human animals include wing shape and bristle count in *Drosophila*; birth weight, temperament and milking ability in cattle; and plumage and beak size in birds. When shown on a graph, the result is a bell-shaped curve (typical of **continuous variation**), which is referred to as a normal distribution.

Height in humans

Height in humans is controlled by about 50 genes or regions of the genome (Figure 7.2.12). Some individual characteristics of height are controlled by genes that include the secretion of thyroid gland hormones and human growth hormones. A deficiency in the amount of these hormones during childhood and puberty can result in stunted growth. Too much of them can cause excessive growth resulting in exceptional height. The greater the number of genes that control a characteristic, the more possible gene combinations exist. This results in a greater number of genotypes for a characteristic and more phenotypes.

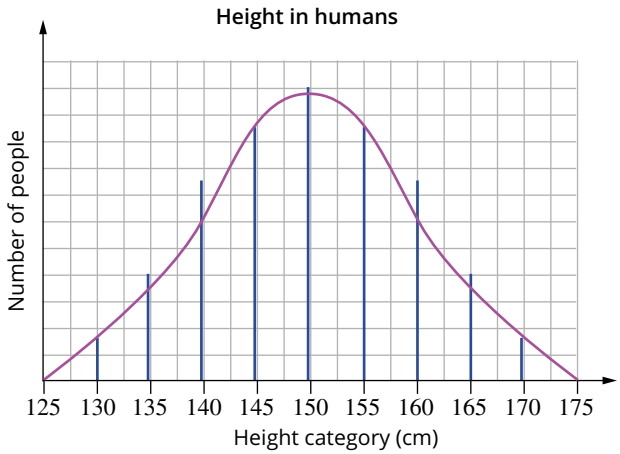


FIGURE 7.2.12 Height in humans is an example of continuous variation resulting from polygenic inheritance.

HISTOGRAMS

Variation in allele frequencies can be represented in the form of a histogram. Figure 7.2.13 shows frequencies for alleles related to a number of conditions in humans. Figures 7.2.11 and 7.2.12 (page 339) show simplified histograms for blood types and height. Frequency histograms like these can represent simplified data where discrete traits are listed on the x-axis and a percentage occurrence in a population is given on the y-axis. For example, Figure 7.2.11 shows that the B blood type occurs in approximately 8% of the sampled population.

Other histograms can be used in industries such as agriculture and biosciences. The histograms presented in Figure 7.2.13 show frequencies for alleles related to the length of maize (corn) ears and the width of maize ear leaves (the leaves that grow around the corn cob). The maize ear leaf is the largest leaf on the plant and is a large photosynthetic region for the plant. Leaf size significantly impacts plant growth and productivity. By studying data such as this histogram, plant scientists can select the best genotypes for crossbreeding or genetic engineering in order to produce superior crops.

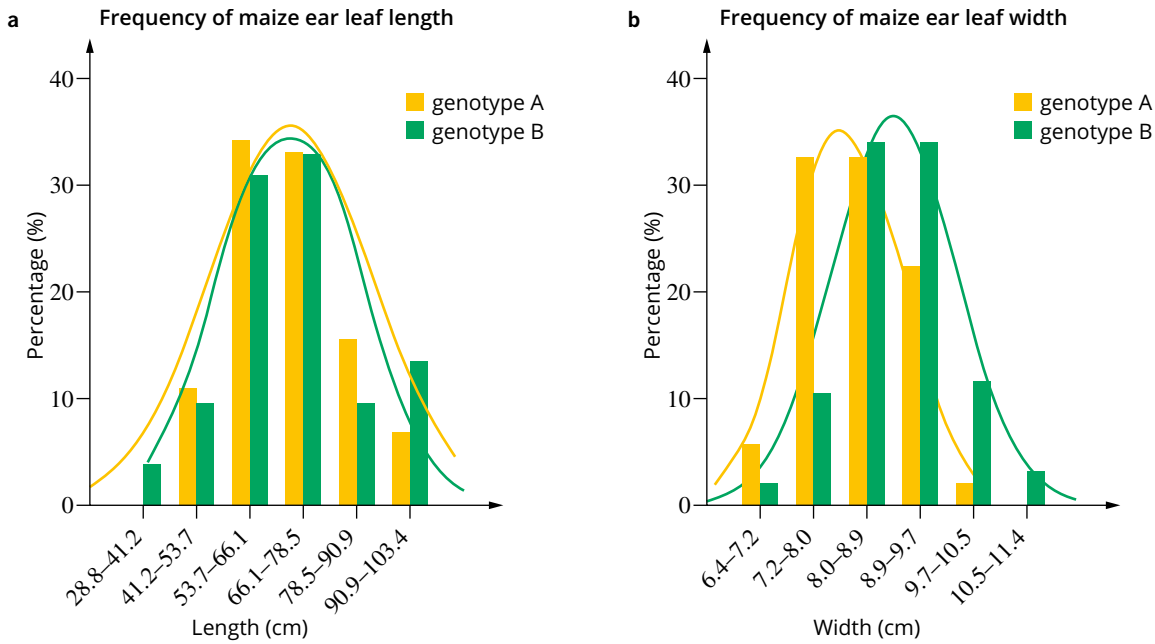


FIGURE 7.2.13 Allele frequency histograms for (a) maize ear length and (b) maize ear leaf width of two genotypes.

7.2 Review

SUMMARY

- Genotype is the combination of alleles at a particular locus.
- An organism that has two copies of the same allele is homozygous for that allele.
- An organism that carries two different alleles is heterozygous.
- Phenotype is an observable characteristic or trait that results from the genotype under the influence of the environment.
- Dominance and recessiveness are properties of alleles and are expressed as dominant or recessive phenotypes.
- A phenotype can be dominant or recessive depending on its appearance in the heterozygote.
  - A dominant phenotype is visible in the heterozygote and one homozygote.
  - A recessive phenotype is only observed in the homozygous condition.
- An italic upper-case letter is used to signify the allele for a dominant phenotype. An italic lower-case letter is used to signify the allele for a recessive phenotype.
- When more than one gene influences a trait, it is called polygenic inheritance.
- Polygenic inheritance causes a wide variety of phenotypes. This is called continuous variation.
- Discontinuous variation occurs when a single gene determines a trait.
- Phenotype is influenced by:
  - genotype
  - interactions between genotype and the environment.
- Fur colour in rabbits, flower colour in hydrangeas and the management of PKU are examples of the way that environment can affect an organism's phenotype.

KEY QUESTIONS

Retrieval

1 Define genotype.

2 Define phenotype.

3 Explain what is meant by a monogenic trait.

4 Recall a polygenic trait in humans.

Comprehension

5 Determine the possible combinations of the alleles *E* and *e* for a particular autosomal gene in an organism with 2*n*. Identify whether each is homozygous or heterozygous.

6 Describe what factors contribute to an individual's phenotype. Give an example.

7 Using an example, explain whether two organisms can have the same phenotype but different genotypes.

8 Explain, using examples, the difference between dominant and recessive phenotypes.

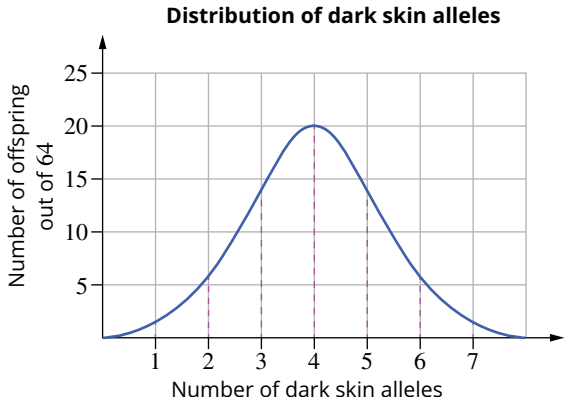
9 Explain why monogenic traits generally show discrete variation.

Analysis

10 From the frequency histogram below:

a Determine the most common allele.

b Calculate the percentage of the sample population that contains the most common allele.



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7.2 Review *continued*

11 Cystic fibrosis (CF) is a genetic disorder that affects the cells that produce mucus, sweat and digestive juices. The gene responsible for causing CF is located on chromosome 7. More than 1500 CF mutations have been identified. For an individual to express CF, they must inherit two mutated CF alleles. The table below shows the frequency of the four most common CF mutations in people living with CF in Australia in 2014.

Frequency of CFTR mutations in people living with CF in Australia, 2014

CFTR mutation	Number of alleles
F508del	4321
G551D	238
R117H	122
G542X	91
other	1298

a Construct a histogram from the table above.

b Determine if CF is a monogenic or polygenic trait.

7.3 Monohybrid crosses

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recall Mendel's principles of inheritance
- choose appropriate symbols for alleles when calculating inheritance patterns
- calculate the outcome of a monohybrid cross
- calculate genotypic and phenotypic ratios
- conduct a test cross
- identify sex-linked patterns of inheritance
- calculate predicted offspring ratios from dihybrid crosses.

Much of what is now understood about natural variation and patterns of inheritance in sexually reproducing organisms was originally gained through the work of Gregor Mendel in the 1860s. Mendel accurately deduced the basic principles of inheritance by studying several inheritable traits in pea plants (Figure 7.3.1), using precise experimentation and careful observations over many years.

In this module, you will learn about the basic principles of inheritance, focusing on autosomal and sex-inked inheritance.

FIGURE 7.3.1 After carefully studying the results of crossing different pea plants in his garden, Gregor Mendel deduced the basic principles of inheritance.

MENDEL'S STUDY OF PATTERNS OF INHERITANCE

Mendel demonstrated that traits are passed from parents to offspring, and that these traits form specific patterns over generations of crossbreeding.

Mendel made several observations in his pea experiments:

- purple flowers were dominant over white flowers
- a round seed shape was dominant over a wrinkled seed shape
- a green pod colour was dominant over a yellow pod colour.

These variant or alternative phenotypes occur because of the various alleles of genes on autosomes. Autosomes are chromosomes that are not involved in sex determination (Module 7.2). One of Mendel's most significant observations was that the offspring of the pea plants did not always have the same phenotype as the parents, and that offspring from the same parents were often different from one another. Mendel hypothesised that hereditary units or 'factors' (now called genes) must have different forms (now called alleles) that separate randomly during the production of gametes. These forms would then unite after fertilisation, with each parent contributing one allele to the offspring. Mendel's hypothesis became known as the Law of Segregation or Mendel's first law.

Dominant phenotypes are expressed if the individual carries at least one allele for the dominant trait. Recessive phenotypes are expressed only if the individual carries two alleles for the recessive trait, or is homozygous recessive.

A **cross** is the intentional breeding of two genetically different organisms that results in offspring that inherit genetic material from each parent. A cross of the traits being studied can be carried out to determine which trait is dominant. A **monohybrid cross** is a cross between two individuals with different alleles at a single locus. Multiple crosses of successive generations can be used to determine allelic dominance.

- The first generation is termed the **parental generation** (P).
- Offspring of the parental generation is termed the first filial generation (**F1 generation**).
- Offspring of F1 generation is called the second filial generation (**F2 generation**).

The phenotypic ratios in the F1 and F2 generations indicate which phenotypes are dominant or recessive. Mendel used monohybrid crosses to discover the dominance relationships of traits in pea plants (Figure 7.3.2).

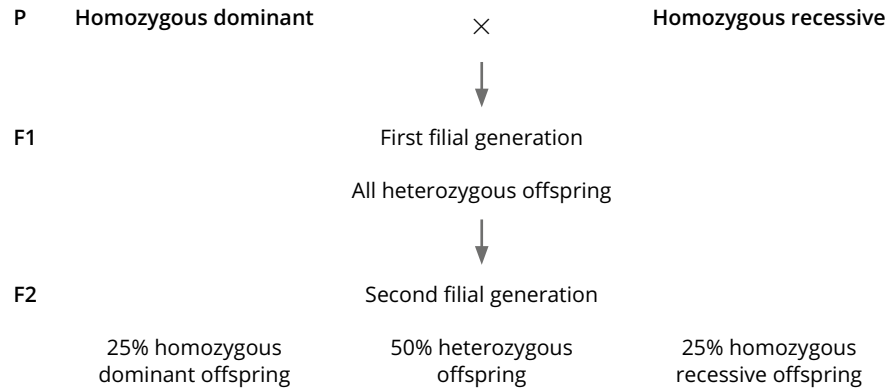


FIGURE 7.3.2 The expected genotypic ratios in the F1 and F2 generations.

AUTOSOMAL DOMINANT INHERITANCE

Autosomal dominant inheritance (complete dominance) refers to a dominant trait that is passed on to offspring via an autosomal gene (Module 7.2). Only one copy of the allele from one parent is needed to express a dominant phenotype.

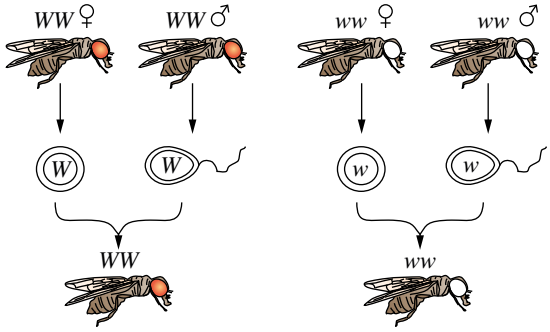


FIGURE 7.3.3 Homozygous genotypes produce only one type of gamete. By crossing homozygotes of the same genotype together, a true-breeding strain can be established.

Parent generation

The inheritance of eye colour in the Australian sheep blowfly is an example of a single gene with two alleles (found on an autosomal chromosome) coding for the trait. In the blowfly, red eye colour is dominant over white eye colour. The homozygous genotypes are *WW* and *ww*. Homozygous genotypes produce only one type of gamete. *WW* individuals produce only *W* gametes and *ww* individuals produce only *w* gametes.

In a cross between two red-eye homozygous (*WW*) individuals, all the offspring would be homozygous *WW* (red eye). As long as *WW* individuals were crossed together, it would be a **true-breeding** red eye strain. Similarly, as you can see on the right side of Figure 7.3.3, crosses between two homozygous white eye (*ww*) individuals would yield a true-breeding white eye (*ww*) strain.

Punnett squares

In 1905, geneticist Reginald Punnett devised a simple method for showing the random combination of gametes and the genotypes of the resulting offspring. In a Punnett square, the alleles of each parent are first written in the top and side cells. By going down each column and across each row, the alleles are combined and written into the remaining cells, showing the expected genotypic ratios of the offspring (Figure 7.3.4)

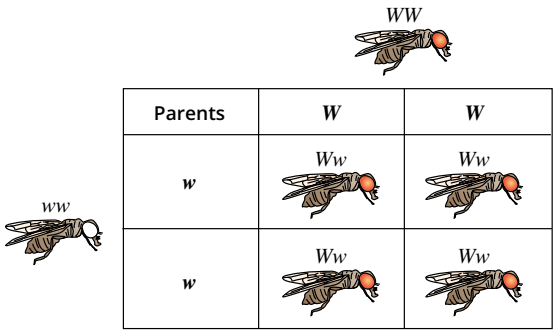


FIGURE 7.3.4 A Punnett square for a cross between two homozygous parents to produce an F1 generation. All F1 individuals are heterozygous.

When choosing symbols for alleles, it is common practice to select one that relates to the dominant phenotype. For example, if the dominant phenotype is grey fur, the dominant allele would be given the symbol *G* and the recessive phenotype would be *g*.

However, the symbols *W* and *w* are traditionally used for eye colour alleles in flies, even though red eye colour is dominant. This is because other genes are involved in eye colour in flies, and the discoverers of this gene named it 'white eye gene'.

Punnett squares make it easy to establish all the possible combinations of alleles carried by the gametes and, therefore, all the possible genotypes of the offspring. This is useful in fields such as animal husbandry and horticulture because it allows breeders to select individuals to cross according to the desired traits of the offspring.

F1 generation

To test the principle of dominance, two true-breeding parents with two different traits can be crossed. This type of cross is known as hybridisation, and the offspring are known as **hybrids**.

In the blowfly example, two true-breeding strains (one with red eyes, *WW*, and one with white eyes, *ww*) can be crossed to produce an F1 generation. The results of the cross can be shown in a Punnett square (Figure 7.3.4).

Each of the offspring in the F1 generation has the heterozygous genotype *Ww*. The phenotype resulting from this genotype is red-eyed. From this, it can be deduced that the red-eye phenotype is dominant over the white-eye phenotype.

F2 generation

The F2 generation is the result of crossing the individuals from the F1 generation. In this example, half of the gametes produced by an F1 individual (*Ww*) will be *W* and half will be *w*. Three different combinations of alleles are possible in the F2 generation: *WW*, *Ww* and *ww* (Figure 7.3.5).

Parents	*W*	*w*
W	*WW*	*Ww*
w	*Ww*	*ww*

FIGURE 7.3.5 A Punnett square of a cross between two F1 individuals to produce an F2 generation.

The Punnett square shows that the F2 genotypic pattern is:

WW : *Ww* : *Ww* : *ww* or 1 *WW* : 2 *Ww* : 1 *ww*

Because red eye colour is dominant over white eye colour, the F2 phenotypic pattern is:

3 red-eyed flies (*WW* : *Ww* : *Ww*) : 1 white-eyed fly (*ww*)

In other words, in the F2 generation, the dominant phenotype is likely to occur in 3 out of 4 crossings, and the recessive phenotype only once.

From this information, it can be determined that the trait that is most common in the natural population is red eyes. The most common trait in the natural population is also known as the **wild type**.

The genotypic pattern 1 : 2 : 1 ratio of the F2 generation resulting from a monohybrid cross occurs for two reasons:

- In meiosis, heterozygous (*Ww*) individuals (both male and female) produce two gametes (a *W* gamete and a *w* gamete). This is because of the separation of pairs of alleles during the formation of reproductive cells.
- Fertilisation occurs at random. A *W* sperm has equal chance of fertilising a *W* egg or a *w* egg, because these eggs are produced in equal frequency. A *w* sperm also has an equal chance of fertilising a *W* egg or a *w* egg. The four equally possible genotypic outcomes are *WW*, *Ww*, *wW*, *ww*.

The Punnett square accounts for both of these factors in demonstrating the possible outcomes of the cross.

Genotypic and phenotypic ratios

Genotypic and phenotypic ratios are used to express the expected frequency of genotypes and phenotypes in the offspring from a genetic cross. Punnett squares are used to calculate the expected outcomes of a cross and the possible genotypes and phenotypes generated in the offspring.

The ratio of genotypes in the offspring is written in the following order:

homozygous dominant : heterozygous : homozygous recessive

The ratio of phenotypes observed in the offspring is written as:

dominant phenotype : recessive phenotype

Genotypic and phenotypic ratios sometimes differ because the dominant or recessive nature of traits means that different genotypes can result in the same phenotype. For example, both *WW* and *Ww* result in red eyes in flies.

Test crosses

It is not immediately obvious whether an individual with a dominant phenotype is homozygous, because it might be either *AA* or *Aa*. Apart from sequencing the gene involved (which is very expensive and time-consuming), the only way to determine this is to do a **test cross**. A test cross involves crossing the individual with another that has the recessive trait and is therefore homozygous. Homozygous individuals produce gametes with one type of allele, whereas heterozygous individuals can produce gametes with two types of alleles.

If the offspring from the test cross all have the dominant phenotype, then both the parents are likely to be homozygous. (It is not possible to be certain because of the random nature of fertilisation.) If the offspring have both dominant and recessive phenotypes, then the parent with the dominant phenotype must also carry a recessive allele and is therefore heterozygous.

Coat colour in guinea pigs

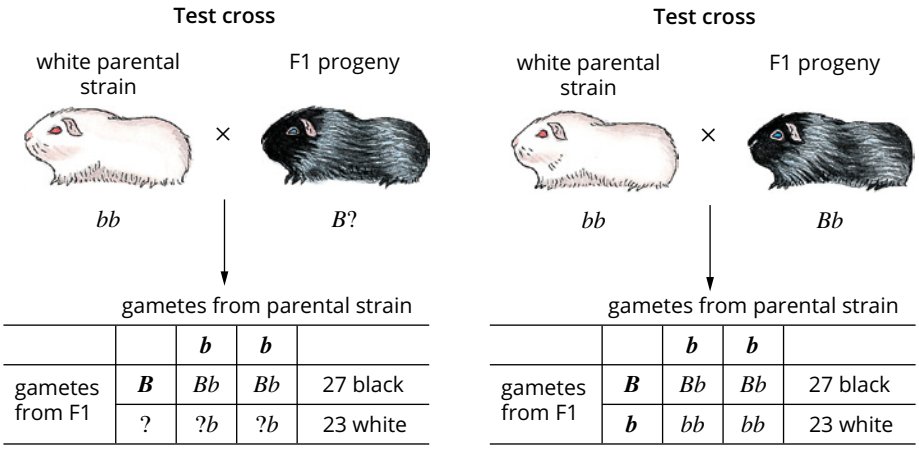
The coat colour of guinea pigs is determined by the alleles of one gene, and black fur is the dominant phenotype. If a true-breeding white guinea pig (*bb*) is crossed with a true-breeding black guinea pig (*BB*), the resulting F1 has black fur (*Bb*). But if the genetic history of a black guinea pig is unknown, its genotype can be determined by crossing the black guinea pig with a white guinea pig, which must be *bb* (homozygous recessive).

Figure 7.3.6 illustrates the test cross that would be carried out. Of the resulting offspring in this example, half are white and half are black. This ratio of 1 : 1 is consistent with the results of a heterozygote crossed with a homozygote if the trait is determined by the alleles of one gene and one trait is dominant. Therefore, the black guinea pig is likely to be heterozygous. If all the offspring of the test cross had black coats (all *Bb*), the black F1 guinea pig would have been shown to be homozygous dominant (*BB*).

The predicted outcome for a cross between heterozygote black guinea pigs is 1 black (*Bb*) : 1 white (*bb*). However, as the diagram shows, the resulting ratio of the test cross was 27 black (*Bb*) : 23 white (*bb*) rather than, for example, 23 : 23 (which is equal to a 1 : 1 ratio). The difference between predicted and observed ratios is due to chance.

Punnett squares provide only the theoretical results of a cross. The actual results from an experiment may be different. Fertilisation can be compared to tossing a coin: for most genes, there are two possible outcomes. If a coin is tossed, there is a 50% chance of getting heads and a 50% chance of getting tails. If the coin is tossed 10 times, you might not get 5 heads and 5 tails, but if it is tossed 1000 times, a heads : tails ratio very close to 1 : 1 would be observed.

Similarly, the more fertilisation events (data) there are in a breeding experiment, the closer the results will be to the theoretical ratio.



		b	b	
gametes from F1	B	Bb	Bb	27 black
	?	?b	?b	23 white

		b	b	
gametes from F1	B	Bb	Bb	27 black
	b	bb	bb	23 white

FIGURE 7.3.6 A test cross between a white guinea pig and a black guinea pig whose genotype is not known.

PA 4.1.3

AUTOSOMAL CO-DOMINANT INHERITANCE

Some traits do not show simple dominance or recessiveness. These are instances in which both alleles are expressed to varying degrees in the phenotype of heterozygous individuals. This is called co-dominance.

Autosomal co-dominance

One example is co-dominance in snapdragons, where in the case of flower colour, neither trait is dominant. In the individual heterozygous for this trait, neither allele is completely expressed and the result is a blending effect of the two phenotypes.

In snapdragon flowers, R_1 represents the red colour allele and R_2 represents the white colour allele. In this case, because neither is completely dominant, upper-case letters and subscripts are used to distinguish the alleles.

Crossing red-flowering snapdragons (R_1R_1 genotype) with white-flowering snapdragons (R_2R_2 genotype) will yield an F1 generation in which all individuals have the genotype R_1R_2 and are pink-flowering (Figure 7.3.7a). If the F1 plants (R_1R_2 × R_1R_2) are crossed, an F2 generation with a 1 : 2 : 1 genotypic ratio (1 R_1R_1 : 2 R_1R_2 : 1 R_2R_2) would be expected (Figure 7.3.7b).

Parents		Red flowers	
		R_1	R_1
White flowers	R_2	R_1R_2	R_1R_2
	R_2	R_1R_2	R_1R_2

Parents		Pink flowers	
		R_1	R_2
Pink flowers	R_1	R_1R_1	R_1R_2
	R_2	R_1R_2	R_2R_2

The heterozygote pink-flowering snapdragon (R_1R_2) can be distinguished from the two homozygotes, red R_1R_1 and white, R_2R_2 due to the co-dominance of both the red and white alleles resulting in a pink-flowering phenotype.

Genotype ratio: 1 R_1R_1 : 2 R_1R_2 : 1 R_2R_2

Phenotype ratio: 1 white : 2 pink : 1 red

This phenotypic ratio of 1 : 2 : 1 (Figure 7.3.7) is different to the 3 : 1 ratio of two phenotypes observed in complete dominance.

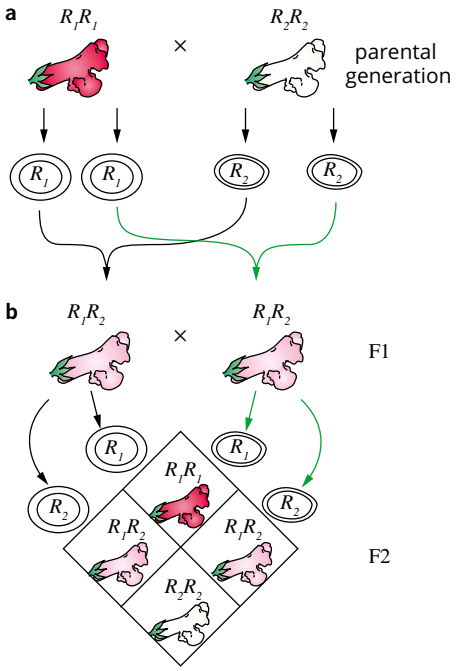
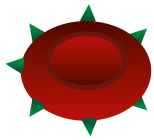
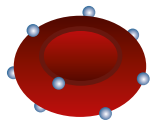
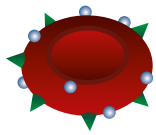
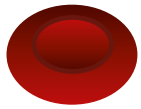


FIGURE 7.3.7 (a) A cross between homozygous red and white snapdragons produces pink-flowering progeny in the F1 generation. (b) If plants from the F1 are then crossed, a phenotypic ratio of 1 red : 2 pink : 1 white would be expected in the F2 generation.

Multiple alleles at a single locus

Blood group systems provide a demonstration of the effects of multiple alleles at the same locus. For example, in the ABO blood grouping system the three alleles are represented as I^A, I^B and i. I^A codes for the A antigen, I^B codes for the B antigen and i does not produce either antigen. The effects of I^A and I^B dominate over i, while the A and B phenotypes co-dominate. Each person carries copies of one or two of these three possible alleles. Table 7.3.1 shows the possible genotypes and phenotypes for the ABO blood group system.

TABLE 7.3.1 Possible genotypes and phenotypes in the ABO blood group system

Genotype	Phenotype (blood group)	Red blood cell
I^AI^A I^Ai	A	
I^BI^B I^Bi	B	
I^AI^B	AB	
ii	O	

From Table 7.3.1 it can be seen that there are six possible genotypes and four phenotypes, with the A and B blood groups both having two possible genotypes.

The possible genotypes and phenotypes of the offspring of a parent with blood type O and a parent with blood type AB can be determined using a Punnett square, as shown below.

Parents		Blood type AB	
		I^A	I^B
Blood type O	i	I^Ai	I^Bi
	i	I^Ai	I^Bi

The F1 generation in this example would be either blood type A or B, but all would be heterozygous.

If a heterozygous individual for blood type A and a heterozygous individual for blood type B were to have children, four possible combinations of blood type are possible, as shown in the following Punnett square.

Parents		Blood type B	
		I^B	i
Blood type A	I^A	I^AI^B	I^Ai
	i	I^Bi	ii

The F1 generation would show all of the phenotypes possible: AB, A, B and O. The important principle illustrated by this example is that phenotypes are not always dominant or recessive. The dominance of a phenotype is always in relation to another phenotype. Thus, phenotype A is co-dominant with B, but dominant to O.

WS 4.1.9

SEX-LINKED INHERITANCE

So far you have examined the inheritance of genes located on autosomes. However, the patterns of inheritance are not the same for genes located on either of the two sex chromosomes. Phenotypes inherited through genes on sex chromosomes are said to be 'sex-linked' and they show **sex-linked inheritance**. It is important to remember that sex chromosomes also carry other genes that are not related to sex determination.

Figure 7.3.8 shows how sex chromosomes are transferred to the offspring, with an equal probability of the offspring being female or male. The XY system determines sex in humans, most other mammals, some insects and some plants. In this system, females are homogametic (XX) and males are heterogametic (XY). The female passes one X chromosome on to her offspring, while the male can pass on either an X or Y chromosome. An X chromosome from the father produces female offspring and a Y chromosome from the father produces male offspring. It is the father's genetic contribution that determines the sex of the offspring. In birds, some fish, some insects and some reptiles, sex is determined by ZW chromosomes. In this system, females are the heterogametic sex (ZW) and males are the homogametic sex (ZZ).

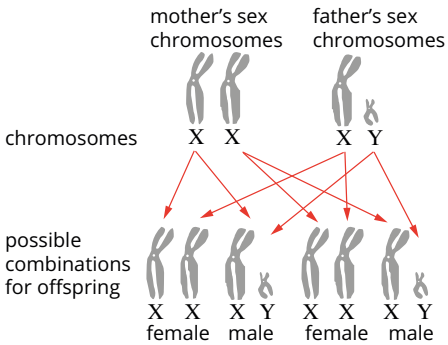


FIGURE 7.3.8 Inheritance of the sex chromosomes in the XY sex-determination system. The outcome of this inheritance is two possible arrangements—XX or XY with half the offspring being female and half being male.

X-linked recessive inheritance

In humans, X-linked recessive traits are predominantly expressed in males, because males carry only one X chromosome. Females carrying an X-linked recessive allele might not express the trait, or show only mild expression. This is because the second X chromosome that females carry could mask the recessive trait. The probability in humans of a female carrying two X-linked recessive alleles is very low.

The pattern of sex-linked inheritance is evident when a **reciprocal cross** is performed. A reciprocal cross is an experiment to investigate the role of parental sex on the inheritance of genotypes. A reciprocal cross involves two crosses: one crossing a male with the trait of interest with a female not expressing the trait (usually homozygous wild type), and another crossing a female with the trait of interest (homozygous) with a male that does not express the trait (usually wild type). If the trait is sex-linked (carried on the X chromosome), the phenotypic ratios of the male and female offspring will be different.

Paralysis in Drosophila

The temperature-sensitive paralytic gene, named after the mutant phenotype, is on the X chromosome of the fruit fly (*Drosophila melanogaster*). A mutant phenotype arises from a genetic mutation that causes phenotypic change from the normal wild type phenotype. Individuals with the mutant allele are paralysed when incubated to a temperature of 29°C, whereas wild type flies show normal behaviour at this temperature. The paralytic phenotype is recessive to the wild type. For this trait, wild type flies move around normally and are not paralysed when the temperature is 29°C.

Alleles are defined differently for sex-linked traits. An *X* is used to indicate that the trait is carried on the X chromosome, and the allele is written in superscript next to the X. In the example of the fruit flies, the alleles can be written as:

X^P = wild type

X^p = paralysis.

As the paralytic phenotype is recessive, females that are homozygous for the mutant allele (X^pX^p) express the mutant paralysis phenotype. Females that are homozygous dominant (X^PX^P) and females that are heterozygous (X^PX^p) both have the wild type phenotype.

As males have only one X chromosome, there are only two male genotypes: X^pY males are paralytic and X^PY males are wild type.

If paralytic females (X^pX^p) are crossed with wild type males (X^PY), all of the F1 male offspring will be paralytic (X^pY) and all of the F1 female offspring will be wild type phenotype (X^PX^p) (Figure 7.3.9a). This pattern of transmission of the mutant phenotype from the female parent to male offspring is characteristic of X-linked recessive inheritance.

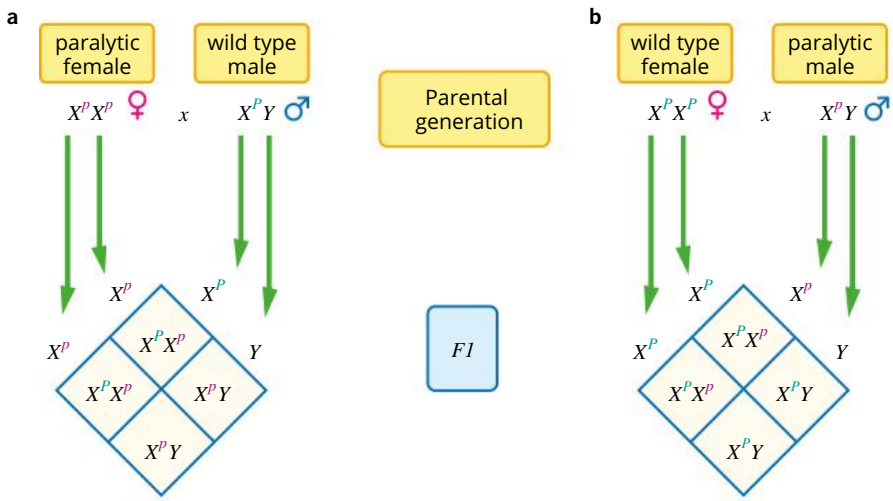


FIGURE 7.3.9 The characteristics of X-linked inheritance are evident in a reciprocal cross. (a) A male receives an X chromosome from the female parent, so males are paralytic (X^pY) and females are wild type (X^PX^p). (b) In the reciprocal cross, both the male and female offspring are wild type.

The reciprocal cross produces a different outcome (Figure 7.3.9b on page 349). If a wild type homozygous female (X^PX^P) is crossed with a paralytic male (X^pY) all of the offspring (male and female) are wild type (X^PX^p and X^PY).

These different outcomes of the reciprocal crosses are characteristic of X-linked recessive inheritance.

Haemophilia in the British royal family

Figure 7.3.10 is a pedigree chart showing part of the family tree of the British royal family (pedigree charts are discussed further in Module 7.4). The chart includes Queen Victoria, whose eighth child, Leopold, was born with haemophilia. Haemophilia is a blood disorder in which blood clotting is slow, resulting in excessive bleeding. It results from a mutation in a gene on the X chromosome that is involved in the production of a blood-clotting protein that controls bleeding.

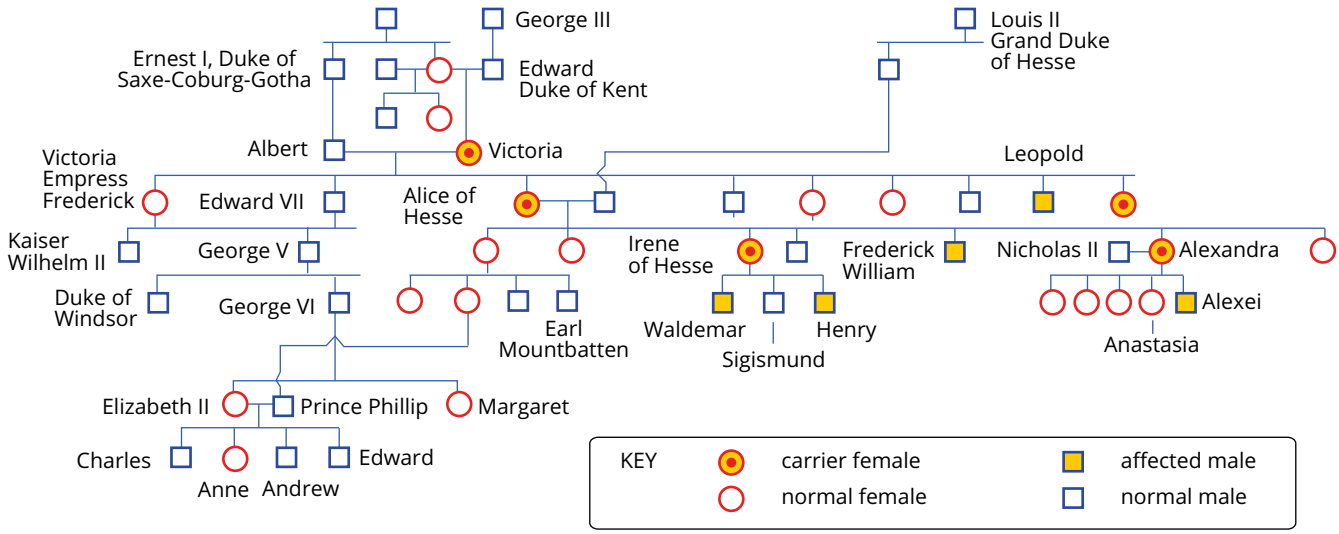


FIGURE 7.3.10 Queen Victoria was a carrier of a mutation that causes haemophilia. The affected gene codes for a blood-clotting protein. She had one copy of the normal allele and one copy of the mutant allele. Some of Queen Victoria's female descendants have been carriers, and some male descendants have had the disease.

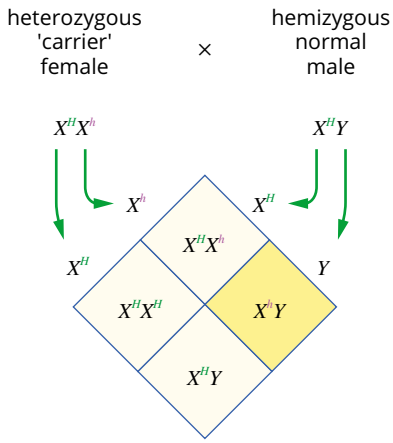


FIGURE 7.3.11 Diagram highlighting the typical situation for the inheritance of X-linked recessive diseases such as haemophilia. A female who is phenotypically normal carries one copy of the allele for the dominant normal phenotype X^H and one for the recessive (mutant) phenotype X^h. All of the female offspring will have a normal phenotype, but there is a 50% chance that each male offspring will inherit the disease.

The incidence of haemophilia in the descendants of Queen Victoria shows the hallmarks of X-linked recessive inheritance. All of the haemophiliacs shown in the tree are male. The female **carriers** of the disease are heterozygous, carrying one haemophiliac allele and one normal allele. Given that the haemophilia phenotype is recessive, carrier females are phenotypically normal. However, because females produce eggs carrying the normal and haemophiliac alleles with equal frequency, and males receive their single X chromosome from the egg, there is a 50% chance that the son of a carrier will have haemophilia.

Through marriage, some of Victoria's phenotypically unaffected daughters who carried this mutation spread haemophilia to other royal families in Europe. For example, Irene of Hesse transmitted the haemophilia allele to her sons Waldemar and Henry, and the normal allele to her other son, Sigismund. This form of haemophilia occurs at a frequency of 1 in 10 000 males and 1 in 100 million females in the general population.

In general, X-linked recessive disorders occur at much higher frequencies in males than females because, in order to be affected, females need to inherit a copy of the allele from both parents (their mother must be a carrier and their father must be affected by the disorder). Males, however, need only inherit one copy of the X-linked allele from their carrier mother (Figure 7.3.11).

X-linked dominant inheritance

X-linked disorders may also display a dominant phenotype. Consider the inheritance of vitamin D–resistant rickets disorder, which causes bone deformities, a pedigree for which is shown in Figure 7.3.12 (pedigree charts are explained in more detail in Module 7.4). The mother of the first generation is heterozygous and affected by the condition. Her children had a 50% chance of having vitamin D–resistant rickets, regardless of whether they were male or female.

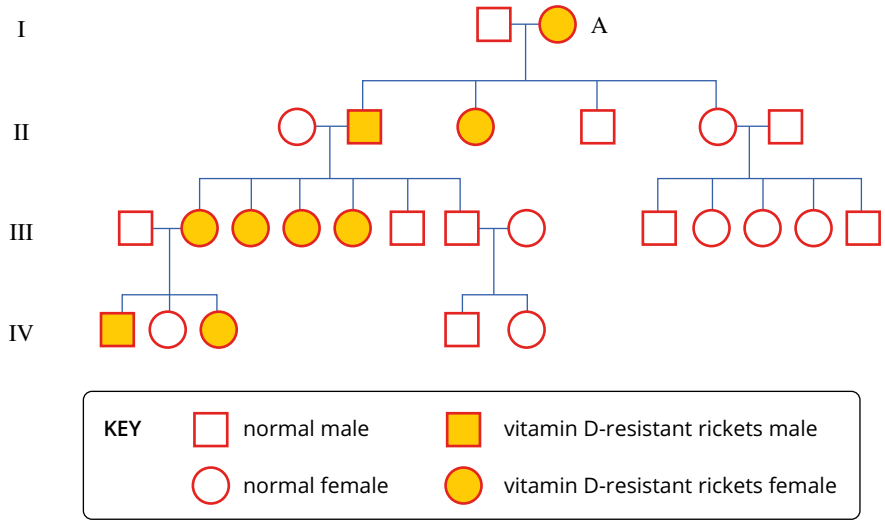


FIGURE 7.3.12 Pedigree chart showing the inheritance of the X-linked dominant condition vitamin D–resistant rickets. The mother (A) of the first generation is heterozygous for the gene that controls the condition.

When a father is affected and a mother is normal (as in the first generation), all female offspring will show the condition, and all male offspring will be normal. This is because female children all receive an X chromosome, which carries the allele for the disease, from their father.

The alleles for this trait could be shown as:

X^D = vitamin D–resistant rickets allele

X^d = normal allele

The following Punnett square shows the pattern of inheritance of offspring of a heterozygous female affected by the vitamin D–resistant rickets allele and a male with the normal allele.

Parents		**Mother**	
		X^D	X^d
Father	X^d	X^DX^d	X^dX^d
	Y	X^DY	X^dY

The possible genotypes of the offspring are:

$X^DX^d : X^dX^d : X^DY : X^dY$

1 : 1 : 1 : 1

The possible phenotypes are therefore:

vitamin D–resistant rickets female : normal female : vitamin D–resistant rickets male : normal male

1 : 1 : 1 : 1

The following Punnett square shows the pattern of inheritance of the offspring of a homozygous unaffected female and a male with the vitamin D–resistant rickets disorder.

Parents		**Mother**	
		X^d	X^d
Father	X^D	X^DX^d	X^DX^d
	Y	X^dY	X^dY

The possible genotypes of the offspring are:

$X^DX^d : X^dY$

1 : 1

The possible phenotypes are therefore:

all females have vitamin D–resistant rickets : all males are unaffected

Male pattern baldness

It is estimated that 80% of hair loss is genetic, and though the causes are not yet well understood, it is known that several genes are involved. Baldness is therefore a polygenic trait.

Male pattern baldness is the most common type of baldness. It affects around 40% of men by the age of 40 and around 60% by the age of 60. Affected males gradually start losing their hair, until eventually they have hair only on the sides and back of the head (Figure 7.3.13).

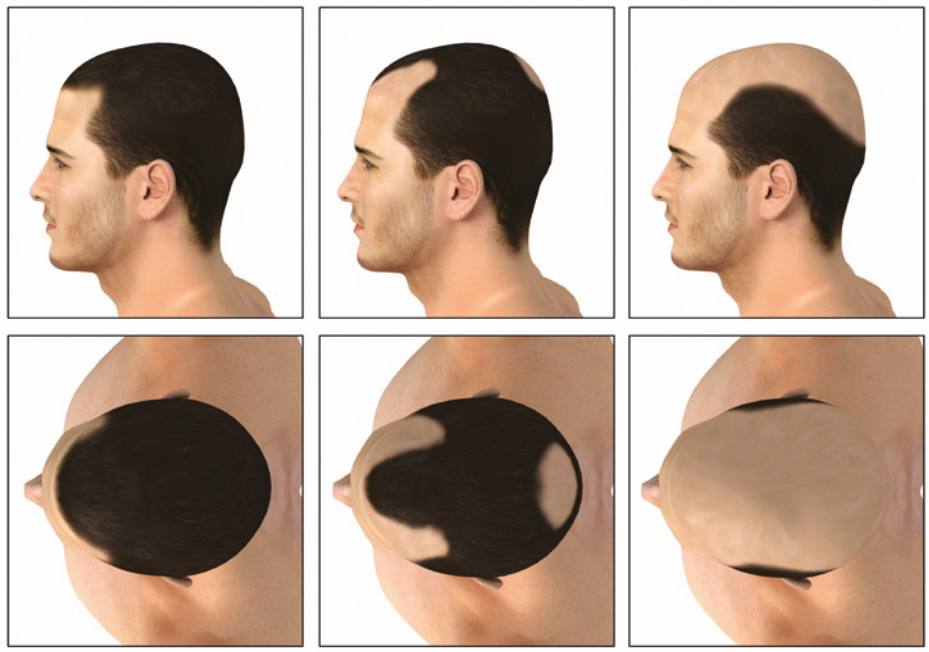


FIGURE 7.3.13 Head of a man showing the change over time of hairline with male pattern baldness. This phenotype can be caused by several genes located on autosomes and the X chromosome.

You may have heard that baldness is inherited from your mother's father. This is because one of the key genes associated with balding is on the X chromosome. If your mother's father has male pattern baldness, then your mother will carry the allele for this characteristic on the X chromosome that she inherited from her father. Because you inherited one of your X chromosomes from your mother, there is a 50% chance that you will inherit the affected X chromosome. However, males are much more likely to express the balding phenotype because females have a second X chromosome to mask the expression of the gene.

Balding can also be passed from fathers to offspring, indicating that autosomal genes must be involved. Two genes on chromosome 20 have been found to contribute to balding. The effects of these genes are neither dominant nor recessive, but have an additive effect—the more copies of the alleles you have, the more likely you are to go bald. Even though these genes are found on autosomes, males are affected more than females. This is because some of the genes are associated with male hormone receptors. This is an example of **sex-limited inheritance**—males and females may have the same genotype but express different phenotypes.

Y-linked inheritance

Compared to the X chromosome, the Y chromosome has few genes. It has only about 72 protein coding genes, compared to 800–900 on the X chromosome. Most of these genes are involved in male sex determination and fertility. Therefore, there are far fewer **Y-linked** traits than X-linked traits.

If a trait is passed from father to son and never observed in females, it is likely to be Y-linked, meaning the gene for that trait is on the Y chromosome. Until recently hairy ears were thought to be controlled by a Y-linked gene, but recent studies suggest there are also autosomal genes involved in the trait.

Sex-limited inheritance

The Y-linked pattern of inheritance is sometimes confused with sex-limited inheritance. Sex-limited traits can only occur in one sex because the feature affected is unique to that sex. Therefore males and females have different phenotypes. For example, complete androgen insensitivity syndrome, in which the fetus is unresponsive to male hormones, can only occur in males, because only males carry the Y chromosome. This means that even if females have the genotype for this syndrome, they cannot express the condition.

7.3 Review

SUMMARY

- The Law of Segregation states that individuals carry pairs of alleles of each gene, which segregate into gametes during meiosis so that each gamete carries one allele of each gene.
- True-breeding strains are homozygous at the locus of interest and produce genetically identical progeny when crossed with each other.
- A phenotypic ratio approaching 3 : 1 will be observed in the F1 generation of a monohybrid cross between two heterozygous individuals for any trait controlled by a single autosomal gene, with two different alleles, controlling a dominant trait.
- A test cross involves crossing an individual displaying the dominant phenotype but unknown genotype with an individual displaying the recessive phenotype(s).
- Test crosses are used to determine whether an individual of dominant phenotype is homozygous or heterozygous.
- A phenotypic ratio approaching 1 : 2 : 1 will be observed in the F2 generation of a monohybrid cross for any trait controlled by a single autosomal gene, with two different alleles, displaying co-dominance.
- Co-dominant inheritance can be seen in ABO blood grouping.
- Phenotypes inherited through the action of genes located on either the X or Y chromosomes show sex-linked inheritance.
- X-linked recessive inheritance shows a pattern of transmission of the mutant phenotype from the female parent to male offspring.
- X-linked dominant inheritance shows a pattern of transmission of the dominant trait from an affected male parent to all female offspring and from an affected heterozygous female parent to 50% of all offspring.
- Y-linked inheritance shows a pattern of transmission of the trait from father to son, and it is never observed in females.

KEY QUESTIONS

Retrieval

1 Explain what an autosomal dominant trait is.

2 Explain the function of a Punnett square in genetics.

3 Define sex-linked inheritance.

4 Recall the term given to the first generation of offspring from a test cross.

Comprehension

5 **a** Define monohybrid cross. Select an example to illustrate your answer.

b In analysing data from a monohybrid cross, explain why you might not expect the results to be exactly the same as the expected ratios.

c Identify what experimental change would increase the probability of the measured data matching the expected ratios.

6 Explain what the following ratios of phenotypes in the F2 generation suggest about inheritance patterns for a particular trait.

a 3 : 1

b 1 : 2 : 1

7 Use the example of eye colour inheritance in the Australian sheep blowfly to answer the following questions.

a Define pure-breeding strain.

b Distinguish between the parental (P), first filial (F1) and second filial (F2) generations.

c Explain why the individuals of the F1 generation are all red-eyed.

d Explain why the phenotypes in the F2 generation occur in the ratio 3 red eye : 1 white eye.

8 Freckles are an inherited trait that result in the formation of spots on fair skin. The gene responsible for freckles is found on chromosome 4 and shows a dominant inheritance pattern.

a Determine the type of inheritance, autosomal or sex-linked.

b Explain how a mother and father who have freckles can have a child that does not have freckles. Determine the probability of this result. Include a Punnett square in your answer.

9 Explain why sex-linked disorders affect males more than females.

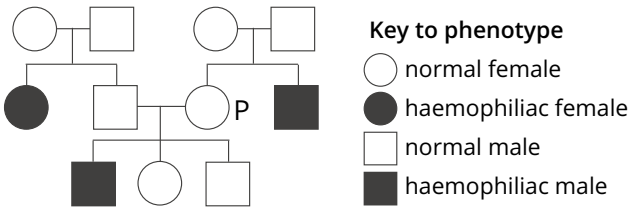
10 Explain why there are fewer Y-linked disorders than X-linked disorders.

11 Determine the probability of the outcome in the following scenario. Blue eyes in humans is a homozygous recessive trait. All other eye colours are dominant to blue. If two non-blue-eyed individuals, who each have one blue-eyed parent, were hoping to have a blue-eyed child together, determine the probability of them having a blue-eyed child.

Analysis

12 Determine the probability of Robert, who has blood type A, and Lee, who has blood type B, having a baby of blood type O. Assume Robert and Lee are heterozygous. Include a Punnett square in your answer.

13 The figure below shows the inheritance of haemophilia in a family. Haemophilia has a recessive X-linked inheritance pattern.



Determine the genotype of individual P. Show your working using a Punnett square and symbolise your answer appropriately.

14 A genetics student undertakes a study of inheritance patterns of feather colour in domestic chickens. The student observes the following:

- Matings between black-feathered adults always result in black-feathered offspring.
- Matings between white-feathered adults always result in white-feathered offspring.
- Matings between black-feathered adults and white-feathered adults produce only blue/grey-feathered offspring.
- Matings between blue/grey-feathered adults result in black, blue/grey and white chickens in a ratio of 1 : 2 : 1.

a Describe the inheritance pattern of this trait. Outline the evidence that leads you to this conclusion.

b Determine how many genes and alleles control this trait. Outline the evidence that leads you to this conclusion.

c Use appropriate notation to set up a model that explains the student's observations.

15 Males of a pure-breeding strain of blowfly with black bodies are crossed to females of a pure-breeding wild type strain. All the offspring are wild type. Male offspring of the reciprocal cross are black-bodied while females are wild type.

a Provide a genetic explanation for these results.

b If offspring of the first cross are mated together, calculate the expected phenotypic ratios of the F2 offspring.
Wild type = X^B and black body = X^b

7.4 Pedigree charts and inheritance patterns

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand the principles of pedigree analysis
- interpret pedigree charts using the recognised symbols
- recognise and identify inheritance patterns from pedigree charts.

FIGURE 7.4.1 Pedigree charts help to determine patterns of inheritance for different traits in families.

Studying the patterns of inheritance in humans has its challenges (Figure 7.4.1). In this module you will learn how patterns of inheritance of alleles across generations of families can be analysed using pedigree charts.

PEDIGREE ANALYSIS

Pedigree analysis can be used to follow the inheritance of traits through a family over a number of generations. Given sufficient data, the likely mode of inheritance can be determined; for example, dominance patterns and whether inheritance is autosomal or sex-linked. When it comes to studying the genetics of humans, pedigree analysis is often the only method available, because:

- Experimental crosses cannot be set up as required.
- The environment in which humans live cannot be controlled experimentally.
- There are strict legal and ethical laws concerning human experimentation.
- Humans tend not to have large families, so there are rarely large numbers of offspring to score.
- Each generation of humans takes many years to reach sexual maturity and produce offspring.

Studying existing families can assist in tracing inheritance of traits. Pedigree analysis is a technique involving studying a family tree for the occurrence of a particular character or trait in one family over a number of generations. In practice, it may be necessary to combine information gained from the pedigree data of several families to determine the most likely mode of inheritance of a particular character.

Pedigrees can be used to determine the pattern of inheritance of particular alleles, as well as the presence of particular alleles within a family and the chances of the allele occurring in offspring.

FIGURE 7.4.2 Pedigree analysis can be used to trace the inheritance of particular traits in animals, such as coat colour in horses.

Pedigree analysis can also be extended to a number of animals that are difficult to study genetically for similar reasons to those for humans. Studbooks are records of parent and offspring phenotypes kept for many years for animals for which ancestry is important. Such animals include recognised dog and cat breeds, thoroughbred horses (Figure 7.4.2) and a number of breeds of farm animals. Zoos also keep studbooks for many of their animals to determine breeding programs for conserving the available genetic variation and to avoid inbreeding.

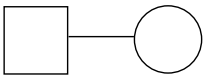
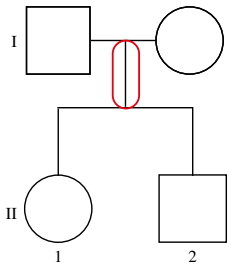
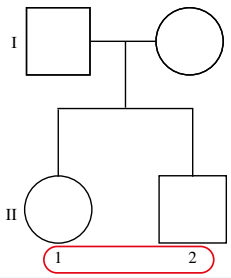
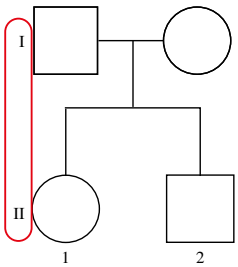
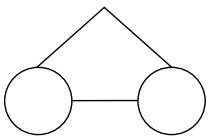
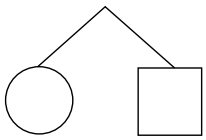
Pedigree charts

Pedigree analysis makes use of pedigree charts to track and organise data. When analysing a pedigree chart, key features in the pattern of inheritance can be used to distinguish between one type of inheritance and another.

Symbols and conventions used for pedigrees

Pedigree charts use a number of standard symbols and conventions, such as those shown in Table 7.4.1. Refer to this table as you work through the pedigree charts in this module.

TABLE 7.4.1 Conventions for pedigree charts

Symbol	Convention
	Circles represent females.
	Squares represent males.
	Shapes are shaded or unshaded to represent the presence of a phenotype for a particular trait. Shaded individuals are affected (expressing the trait) and unshaded individuals are unaffected.
	A carrier of an autosomal trait is shown with half of the symbol shaded.
female carrier	A carrier of an X-linked trait is shown with a dot in the centre of the symbol. Carriers of X-linked traits can only be female.
	A horizontal line represents a cross between the individuals.
	A vertical line represents a link from parents to offspring.
	Individuals are numbered from left to right (if required).
	Generations are represented with roman numerals (if required), with the first generation in the pedigree being generation I.
	Identical twins are joined with a closed triangle.
	Non-identical twins are joined with a triangle open at the bottom.

RECOGNISING INHERITANCE PATTERNS

Autosomal inheritance

Autosomal recessive inheritance

Autosomal recessive inheritance of a trait is likely if two parents do not have a particular phenotype but one or more of their offspring does. Figure 7.4.3a shows the inheritance pattern of a particular trait in a family. Shaded individuals are affected (that is, they express the trait), and unshaded individuals are not affected.

For this exercise, it may be assumed that the inheritance of the trait is not sex-linked. Consider the section of the pedigree chart highlighted by the red square. This shows that:

- the cross between individuals II-3 and II-4 resulted in three offspring
- individuals II-3 and II-4 are unaffected
- offspring III-1 is female and unaffected, III-2 is male and unaffected, and III-3 is female and affected.

The parents both contribute one allele each for the trait to III-3 (white), so both parents must carry the allele responsible for the trait. Since the parents are both unaffected, both must be heterozygous. The trait is therefore autosomal recessive.

Another indicator of autosomal recessive inheritance is that the trait skips generations (that is, it does not appear in every generation). However, not skipping a generation does not rule out autosomal recessive inheritance.

Once the form of inheritance is determined, it is possible to revise the tree (Figure 7.4.3b) and then work out the genotypes of the individuals in the pedigree. First, a symbol should be allocated for the two alleles. In this particular example:

- *F* can represent the allele for freckles (dominant trait)
- *f* can represent the allele for no freckle (recessive trait).

Because individuals II-3 and II-4 are carriers, the symbols representing them can be half shaded in. Individual I-2 is also a carrier, and so should also be half shaded in.

III-3 (no freckles) must be *ff*, both parents must be heterozygous (*Ff*) and individuals III-1 and III-2 must be heterozygous (*Ff*) or homozygous (*FF*).

An example of autosomal recessive inheritance in humans is haemochromatosis, a disorder in which too much iron accumulates in the body, leading to tissue damage.

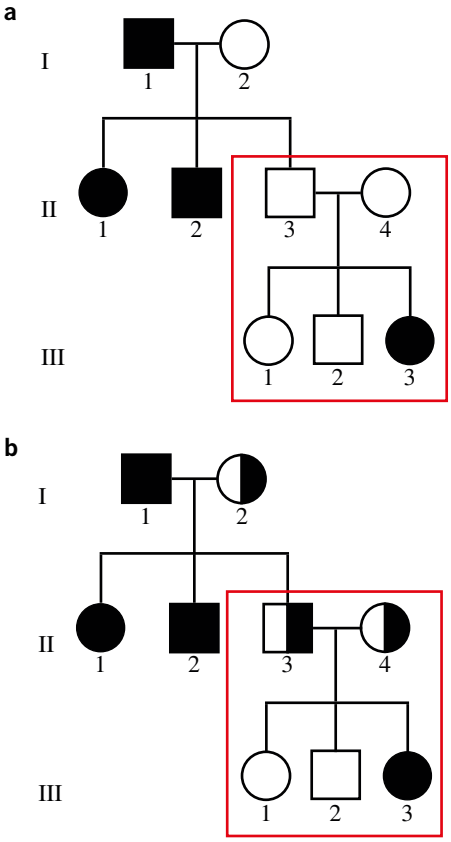


FIGURE 7.4.3 Pedigree charts showing an example of autosomal recessive inheritance. Chart (a) shows before pedigree analysis; chart (b) shows the same chart after inheritance patterns have been analysed.

FIGURE 7.4.5 Wrinkled peas (left) and round peas (right).

Autosomal dominant inheritance

Autosomal dominant inheritance of a trait is likely if both parents show the trait but one or more of their offspring do not show the trait. The pedigree chart in Figure 7.4.4 shows the inheritance pattern of round and wrinkled pea shapes in pea plants (Figure 7.4.5). The shaded individuals represent round peas and the unshaded individuals represent wrinkled peas.

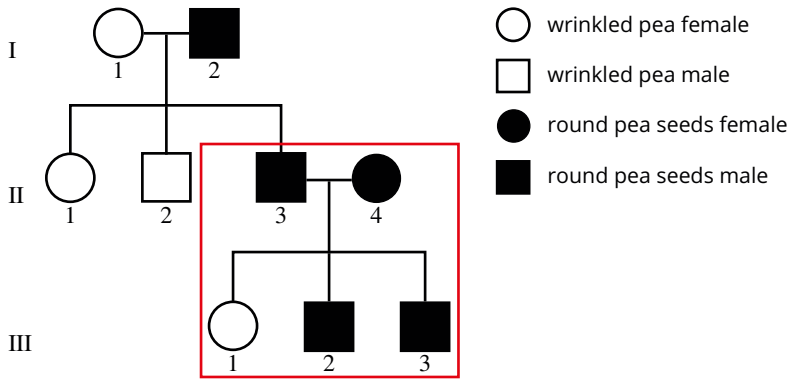


FIGURE 7.4.4 Pedigree chart showing an example of autosomal dominant inheritance.

Again, it may be assumed for this example that the trait is not sex-linked. Consider the section of the chart highlighted by the red square. This shows that:

- II-3 and II-4 produce three offspring
- both II-3 and II-4 are affected
- offspring III-1 is female and unaffected, and III-2 and III-3 are male and affected.

Because both II-3 and II-4 contribute one allele each to III-1, each must carry the allele for the unaffected phenotype. Since each parent carries the wrinkled allele, they must both be heterozygous. As they are heterozygous and show the round phenotype, their phenotype (round) must be dominant.

Other indicators of autosomal dominant inheritance are that the trait may be seen in all generations, and individuals showing the trait must have at least one parent showing the trait.

Examples of autosomal dominant inheritance in humans include Huntington's disease and neurofibromatosis type 1. Examples in other animals include the hornless trait in cattle.

Sex-linked inheritance

In the previous examples of autosomal inheritance, it was assumed that inheritance was autosomal. However, when investigating inheritance it is important to check whether the inheritance might be sex-linked, because similar patterns can occur in both types.

Colour blindness is usually caused by an inherited genetic defect in the light-sensitive pigments of the eye (Figure 7.4.6). Up to 8% of the male population is affected, and 10 times more men have colour blindness than women.

The inheritance pattern of colour blindness in humans can be examined by studying the pedigree of a family in which some individuals are colour blind. By convention, if a mating partner is not shown in the pedigree, he or she is not affected by the phenotype (in this case, not colour blind).

Differences in the incidence of the trait between males and females and the frequency of the trait across generations are two inheritance patterns that help determine whether a trait is sex-linked or autosomal.

In the pedigree chart shown in Figure 7.4.7 only the males are colour blind. This demonstrates that the colourblind trait is not dominant and is potentially sex-linked (X-linked). Y-linked alleles affect only males and are very rare, and the phenotype will be present in every male offspring. Because of these factors, colour blindness cannot be a Y-linked trait.

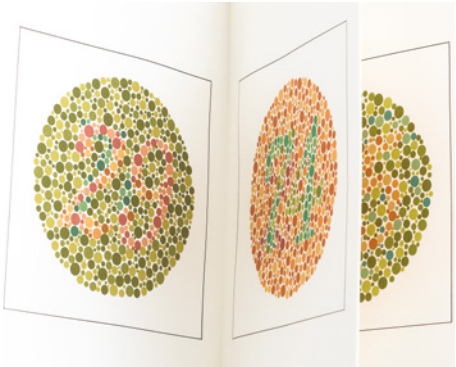


FIGURE 7.4.6 Colour blindness test. People suffering from red–green colour blindness cannot distinguish the numbers.

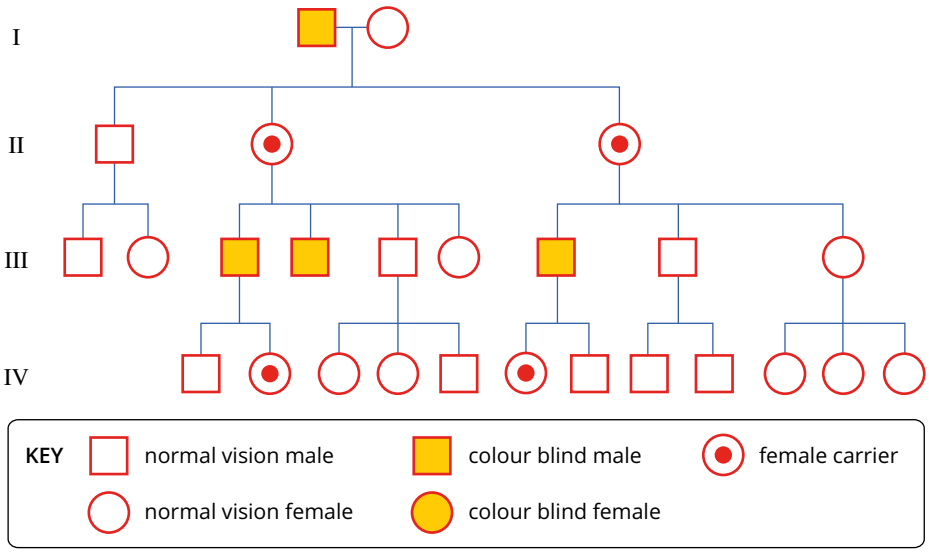


FIGURE 7.4.7 Pedigree chart of a family in which colour blindness is present.

X-linked recessive inheritance

The pedigree chart in Figure 7.4.7 (page 359) also indicates that the inheritance of colour blindness is more likely to be X-linked recessive than autosomal recessive, because X-linked recessive traits affect more males than females. This is because males only inherit one X chromosome, while females inherit two. This second X chromosome has a masking effect on recessive alleles, resulting in females carrying the affected allele but not expressing the phenotype. In order for females to be affected by X-linked recessive traits, they must carry two copies of the allele, one on each X chromosome.

X-linked dominant inheritance

Traits that are X-linked dominant are rare and affect more females than males. This is because females inherit two X chromosomes and so have twice the chance of inheriting an affected X chromosome compared to males, who inherit only one.

Evidence of X-linked dominant inheritance is seen in a pedigree in which affected males have daughters who are all affected and sons who are not affected. This is because daughters inherit their father's only X chromosome, while sons inherit their father's Y chromosome. If the X chromosome carries an allele for a dominant trait, then the daughter will express its phenotype. X-linked dominant inheritance can be seen in Figure 7.4.8.

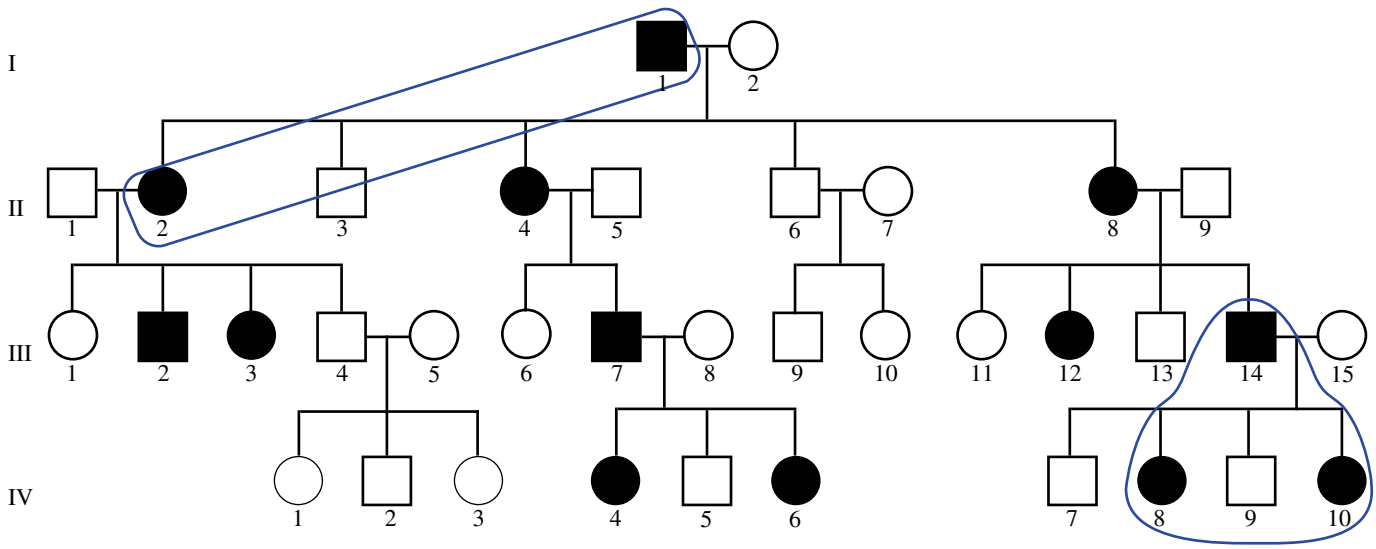


FIGURE 7.4.8 Pedigree chart showing an example of X-linked dominant inheritance. The circled areas of the chart show that daughters of affected males are also all affected.

Males I-1, III-7 and III-14 have daughters who are all affected and sons who are unaffected. Because females pass on one of their two X chromosomes to both their daughters and sons, there is a 50% chance that they will pass on an X-linked dominant trait to their offspring. This pattern of inheritance is seen in female individuals II-2, II-4 and II-8, who have both affected and unaffected daughters and sons.

An example of X-linked dominant inheritance in humans is Rett syndrome (RTT), a rare genetic neurological disorder of the grey matter of the brain. Another example is vitamin D–resistant rickets (Module 7.3).

Y-linked inheritance

Male offspring inherit their father's Y chromosome, so any alleles carried on this chromosome will be passed on from father to son. The phenotype of Y-linked disorders is, therefore, seen in fathers and all their sons. Females are never affected by Y-linked traits because they do not inherit a Y chromosome. As there is only one Y chromosome (hemizygous), and thus only one allele present, the general principles of dominant and recessive inheritance do not apply.

A Y-linked trait is likely if:

- only males are affected
- all male offspring are affected
- the trait is observed in every generation in which males are born.

This pattern of inheritance can be seen in Figure 7.4.9. Because the Y chromosome has far fewer genes that encode for proteins compared to the X chromosome, Y-linked inheritance of disorders is relatively rare.

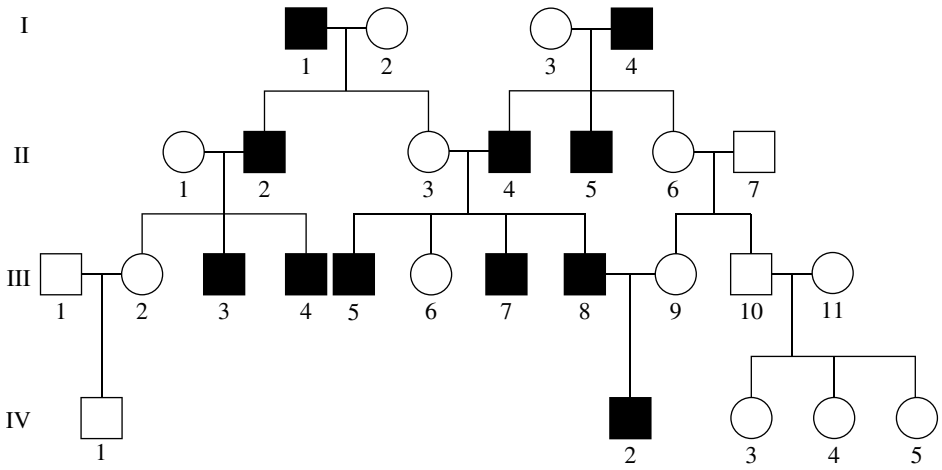


FIGURE 7.4.9 Pedigree chart for a trait with Y-linked inheritance. This pattern of inheritance is evident because only males are affected and the trait is present in males in every generation.

Ruling out sex-linked inheritance

Consider the pedigree chart for a trait in humans shown in Figure 7.4.10. It is possible to rule out sex-linked inheritance in this pedigree by looking for a pattern that will rule out each type of sex-linked inheritance in turn. (If no such pattern can be found, then that type of inheritance cannot be ruled out.)

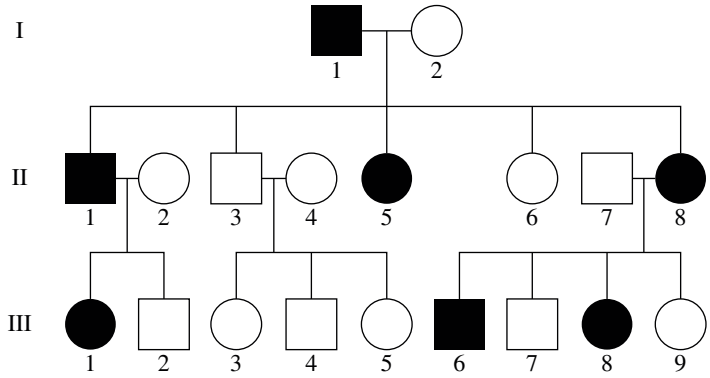


FIGURE 7.4.10 Pedigree chart for an inherited trait in humans.

Ruling out X-linked recessive inheritance

In order for a trait to be X-linked recessive, affected mothers must have affected sons. In Figure 7.4.11, we can see that the affected female II-8 has two sons, one affected by the trait (III-6) and the other unaffected (III-7). If the trait was X-linked recessive, the mother would have to carry the allele on both her X chromosomes (X^hX^h) to be affected. The mother contributes one of these chromosomes to her offspring, so both sons would have to receive a chromosome with the affected allele. But one son (III-7) does not show the trait (and therefore did not receive the affected allele), so X-linked recessive inheritance cannot be involved.

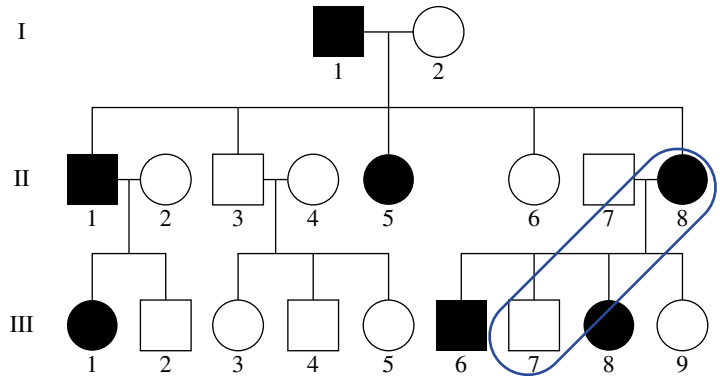


FIGURE 7.4.11 Ruling out X-linked recessive inheritance.

Ruling out X-linked dominant inheritance

If a trait is X-linked dominant, every daughter of an affected male will be affected. This is because the daughters must receive an X chromosome from their father, and the father has only one X chromosome. In Figure 7.4.12, male I-1 has the trait but one of his daughters (II-6) does not. X-linked dominant inheritance therefore cannot be involved in this pedigree.

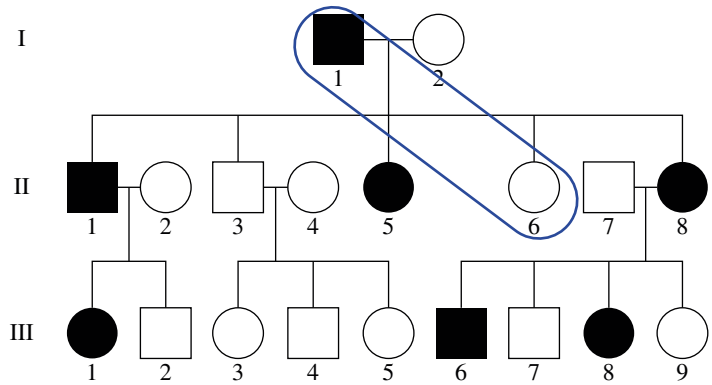


FIGURE 7.4.12 Ruling out X-linked dominant inheritance.

Ruling out Y-linked inheritance

If a trait is Y-linked, only males are affected (because females lack a Y chromosome) and affected fathers pass the trait on to all their sons. In Figure 7.4.13, some females have the trait, and not all fathers with the trait passed it on to their sons (e.g. male I-1 and son II-3; also II-1 and III-2). Y-linked inheritance is therefore not involved in this pedigree.

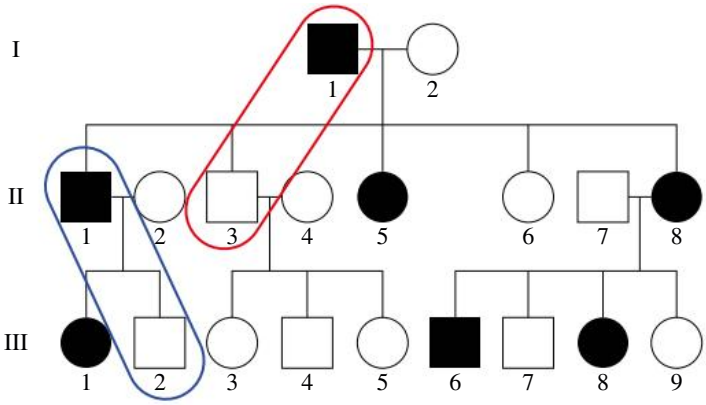


FIGURE 7.4.13 Ruling out Y-linked inheritance.

After ruling out X-linked recessive, X-linked dominant and Y-linked inheritance, it can be concluded that the trait shown in the previous figures must be the result of autosomal inheritance. Although the trait is observed in every generation, which often indicates dominant inheritance, in this case the trait could be autosomal dominant or autosomal recessive inheritance. Further information would be needed to determine which is involved in this pedigree.

STEPS IN PEDIGREE ANALYSIS

A pedigree analysis should be carried out in a methodical series of steps to determine the pattern of inheritance. These steps are outlined below and illustrated in Figure 7.4.14.

Step 1

Determine whether the condition is sex-linked.

a Are mostly males affected? If only males are affected, and the trait passes from father to son in every generation, inheritance is most likely Y-linked.

b Do affected daughters have an affected father? If all daughters of affected males are also affected, the trait is likely to be X-linked dominant.

c Do affected mothers have affected sons but not affected daughters? If so, inheritance is most likely X-linked recessive.

d If you answered 'no' to either question in (b) and (c), go to step 2.

Step 2

Look for two affected/unaffected parents that have a child with a different phenotype.

a If two unaffected parents have an affected offspring, inheritance is autosomal recessive.

b If two affected parents have an unaffected offspring, inheritance is autosomal dominant.

WS 4.1.7 PA 4.1.4

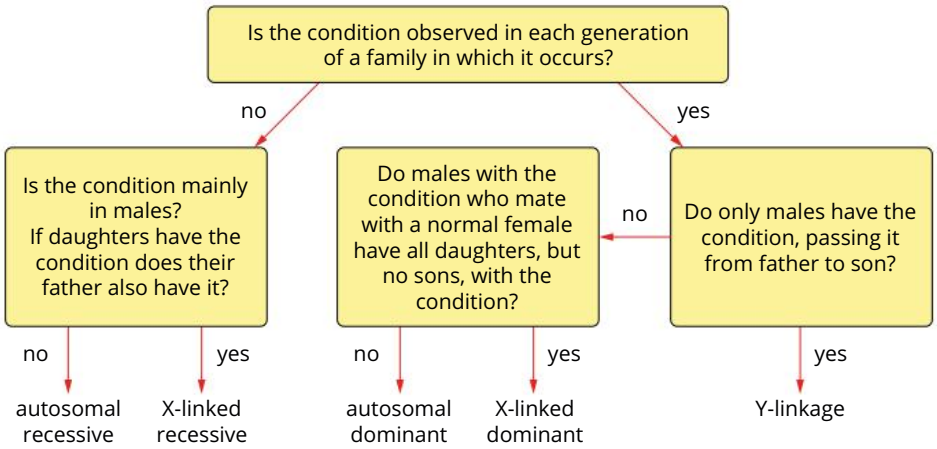


FIGURE 7.4.14 Flow diagram for pedigree analysis of simple modes of inheritance.

7.4 Review

SUMMARY

- Pedigree analysis is a technique of looking through a family tree (of humans or other organisms) for the occurrence of a particular characteristic in one family over a number of generations.
- Pedigrees can be used to determine the likely mode of inheritance, such as dominance patterns and whether inheritance is autosomal or sex-linked.

Autosomal recessive

- Both sexes display the trait in equal numbers in a pedigree.
- Offspring of unaffected parents have a 25% chance of being affected.
- Affected individuals are homozygous.

Autosomal dominant

- Both sexes display the trait in equal numbers in a pedigree.
- One parent must be affected to have an affected offspring.
- Two affected parents with an unaffected offspring indicate dominance: the parents need only one dominant allele to express the trait, so their offspring may inherit their unaffected alleles.
- The trait is observed in each generation.

X-linked recessive

- The trait is rare within the pedigree, but males are more affected than females.
- Affected fathers do not pass the trait on to their sons, so the condition can skip generations.
- Females can be carriers and not show the condition; females pass the trait on to their sons.

X-linked dominant

- Males and females are affected (often more females than males).
- All affected sons have an affected mother.
- All affected daughters have an affected father.
- The trait is observed in each generation.

Y-linked

- Only males are affected, not females.
- Fathers pass the trait on to their sons.
- The trait is observed in each generation.

KEY QUESTIONS

Retrieval

1. Recall which symbol is commonly used to represent an affected male in a pedigree chart.
2. Determine whether a female symbol would be shaded in the case of a Y-linked disease.
3. Recall why Y-linked disorders are rare.

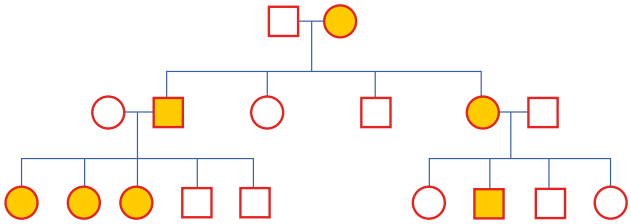
Comprehension

4. State the type of inheritance that is described as being ‘carried on the X chromosome’ and ‘occurs more in males than females’.
5. Explain why males are more likely to exhibit the phenotype of an X-linked trait than females.
6. Explain why Y-linked traits are not considered to be recessive.

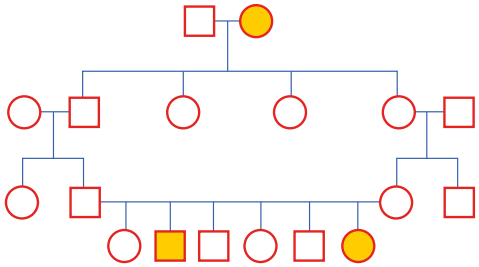
Analysis

7 Determine, with explanation, the possible modes of inheritance for the following two pedigrees.

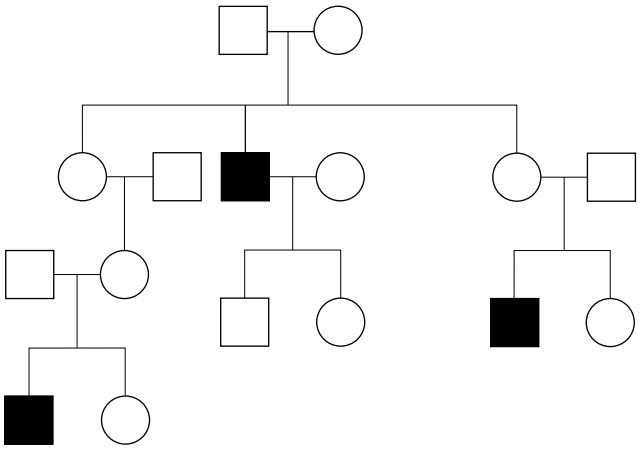
a



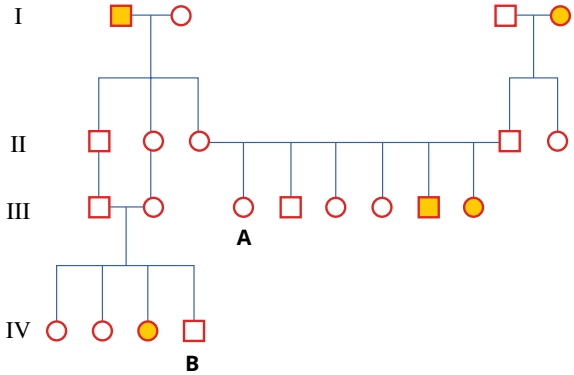
b



8 Interpret the type of inheritance shown in the pedigree chart below.



9 The following pedigree chart shows the inheritance of albinism.



a Justify the most likely mode of inheritance for this condition.

b If III-A mates with IV-B, calculate the chance that an offspring will have the condition. Include a Punnett square in your answer.

7.5 Linked genes, multiple crosses and crossing over

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recall the principle of independent assortment
- explain the concept of filial generations
- calculate crosses containing linked genes.

The crosses discussed so far relate to one trait; for example, the colour of a flower. In this module, you will learn about the inheritance of two characteristics as either independent or linked, and the biological consequence of crossing over for linked genes.

INDEPENDENT ASSORTMENT

Mendel's second law of inheritance, the **Law of Independent Assortment**, states that the alleles of a gene controlling one trait assort independently of alleles of another gene controlling a different trait. This can be illustrated by considering crosses involving two genes that affect two distinct traits.

FIGURE 7.5.1 *Drosophila melanogaster.*

You can cross true-breeding strains of flies that differ for two traits: eye colour and body colour and then conduct a **dihybrid cross** ('di' meaning two). The two traits in this example are eye colour and body colour in *Drosophila melanogaster* (Figure 7.5.1).

The eye-colour gene in this example is the yellow eye gene. (This is a different gene from the white eye colour gene discussed in Module 7.2, and is located on a different chromosome). The yellow eye gene is autosomal and the alleles are *Y* and *y*. The wild type red eye phenotype (genotypes *RR* and *Rr*) is dominant and the yellow eye phenotype (genotype *rr*) is recessive.

The second trait is body colour and the gene in this example is called brown body. It is an autosomal gene with two alleles *B* and *b*. The wild type green body phenotype (genotypes *BB* and *Bb*) is dominant and brown body phenotype (genotype *bb*) is recessive.

The eye-colour and body-colour genes are on different chromosomes.

Dihybrid cross

F1 generation

In a dihybrid cross, a cross is first set up between a true-breeding red eye, green body (*RR*, *BB*) strain and a yellow eye, brown body (*rr*, *bb*) strain. The homozygous *RR*, *BB* strain will produce gametes carrying only the *R* and *B* alleles and the homozygous *rr*, *bb* strain will produce only *r* and *b* alleles. The gametes fuse to produce F1 progeny, which are heterozygous for both genes (*Rr*, *Bb*). Following meiosis, a gamete will end up with any of four possible combinations of alleles: *RB*, *rb*, *rB* or *Rb*.

> The Law of Independent Assortment states that the alleles of genes that code for different traits are inherited independently from each other.

During gamete formation in F1, the chance of a sperm or egg cell containing a *Y* allele is 0.5 (because half of the gametes contain a *Y* allele). The chance that a gamete will contain a *B* allele is also 0.5. Therefore, the chance of the gamete being *YB* is $0.5 \times 0.5 = 0.25$. The probability of each of the other three possible gametic combinations is also 0.25.

This is because the segregation of alleles of a gene on one chromosome in meiosis is independent of the segregation of alleles of a gene on another chromosome. The homologue (matching chromosome) carrying the *Y* allele can move to either pole, as can the homologue carrying the *y* allele. These homologues move independently of the chromosomes that carry the *B* and *b* alleles (Figure 7.5.2). This is the Law of Independent Assortment of genes.

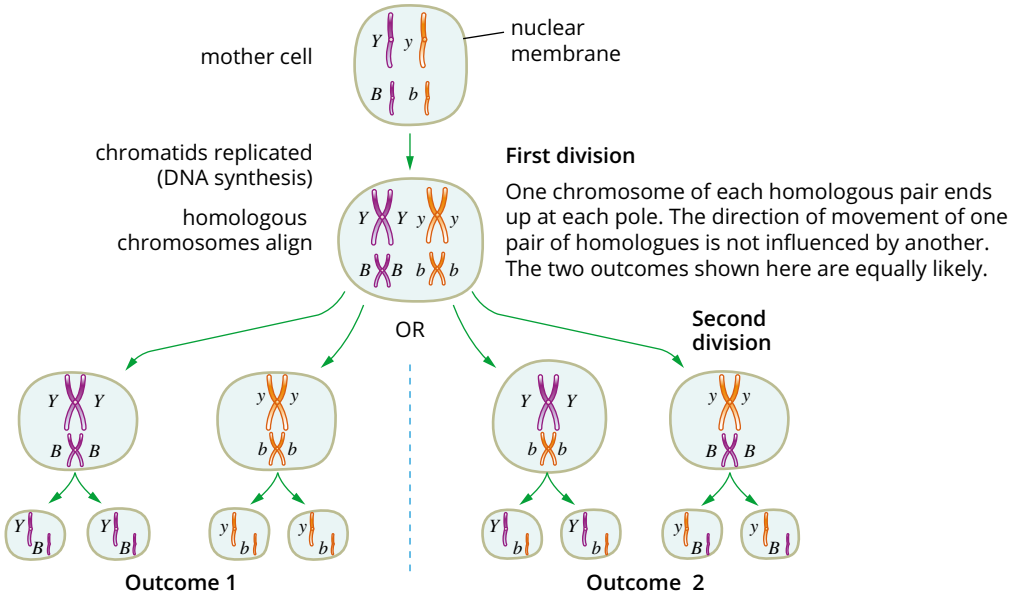


FIGURE 7.5.2 New combinations of alleles result from cells dividing by meiosis. Two genetic loci are shown, each on separate chromosomes within the cell nucleus. Daughter cells produced by meiosis may have any of four possible combinations of alleles.

F2 generation

The heterozygotes generated in the F1 can be crossed together (a dihybrid cross) to produce an F2 generation. The F1 generation produces *YB*, *Yb*, *yB* and *yb* gametes in equal frequency. Figure 7.5.3 (page 368) shows that the expected ratio of phenotypes in the F2 generation is:

- 9 red eye, green body
- 3 red eye, brown body
- 3 yellow eye, green body
- 1 yellow eye, brown body.

If these crosses were actually performed, the phenotypic ratio in the F2 generation should be close to the 9 : 3 : 3 : 1 ratio. There would be some difference between the expected and observed phenotypic ratios due to sampling error. The larger the number of F2 progeny scored, the closer the result will be to the 9 : 3 : 3 : 1 ratio.

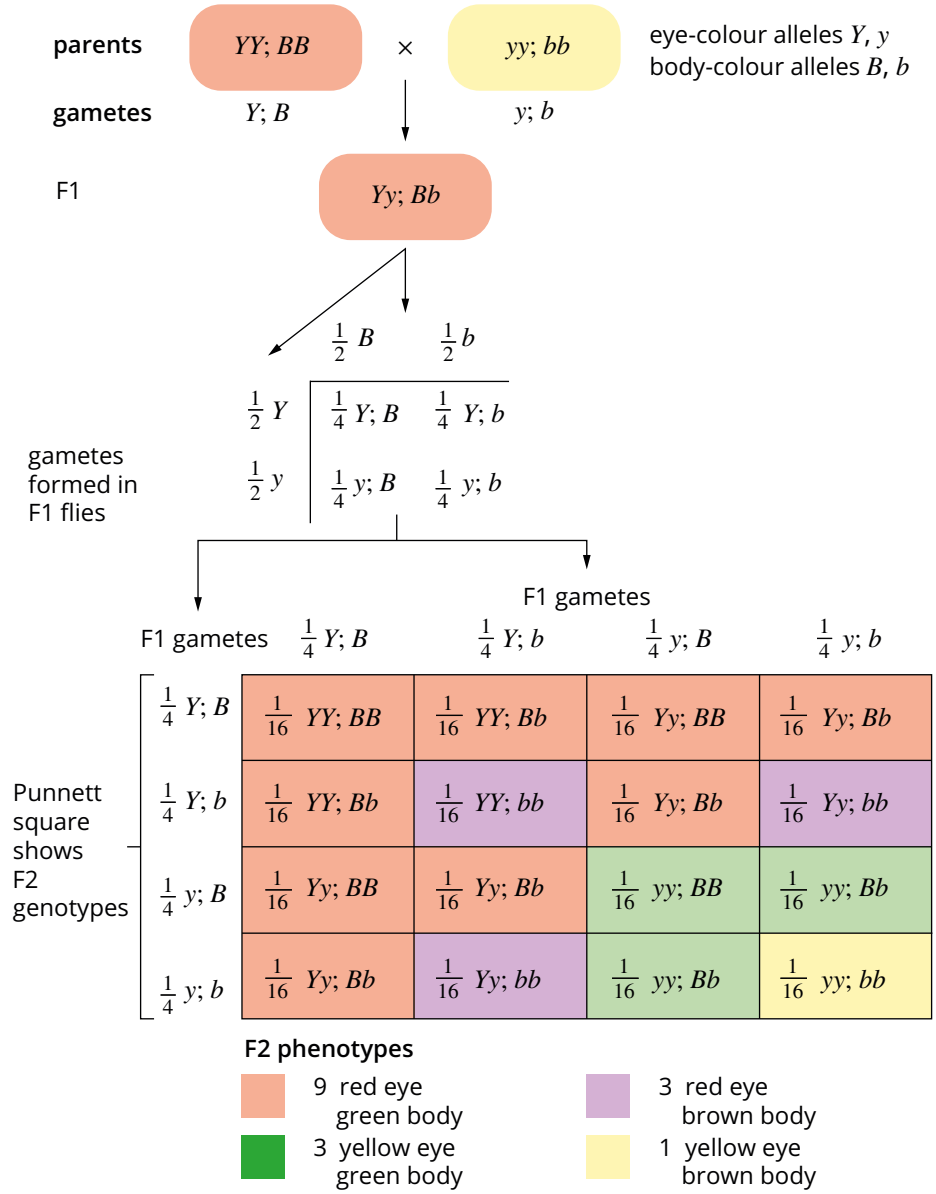


FIGURE 7.5.3 Punnett square showing the genotypes and phenotypes of the F2 progeny.

A phenotypic ratio approximating 9 : 3 : 3 : 1 will be observed in the F2 generation of a dihybrid heterozygous cross if the following five conditions apply:

- the two genes control two distinct traits
- there are two alleles for each of the genes
- one phenotype is dominant for each trait
- both genes are on autosomes
- the two genes assort independently.

In this example, independent assortment occurs because the two genes are on different chromosomes. However, you will learn later in this module that independent assortment can occur via another mechanism.

WS 4.1.6

LINKED GENES

Gregor Mendel's Law of Segregation and Law of Independent Assortment are the cornerstones upon which our current understanding of inheritance is built. Since the rediscovery of Mendel's work 16 years after his death, scientists have continued to refine and extend these laws or principles to explain new and unexpected aspects of heredity, and more complex patterns of inheritance.

Although many traits are inherited in accordance with Mendel's laws, this is not always the case. The exceptions occur when two or more genes are located on a single chromosome and are inherited together. This is known as **linkage**, and is another key principle of inheritance. The closer the genes are, the more likely they are to be inherited together, or 'linked'. But linkage is not always certain because of crossing over, which occurs during meiosis.

The consequences of gene linkage for phenotypes in offspring can be seen in the following example of maize seed shape and colour. These phenotypes are encoded by autosomal genes, each with two alleles.

- Seed colour: orange (genotypes *OO* and *Oo*) is dominant to white (genotype *oo*)
- Seed shape: round (genotypes *RR* and *Rr*) is dominant to flat (genotype *rr*)

A standard dihybrid cross was conducted, starting with two true-breeding parents, one with orange round seeds (genotype *OORR*) and the other with white flat seeds (genotype *oorr*). The F1 offspring all showed the dominant phenotypes (phenotype: orange, round seeds; genotype: *OoRr*).

If the two genes assort independently, the expected genotypes and phenotypes in the F2 generation can be predicted with a Punnett square for a cross between two F1 plants with orange, round seeds (*OoRr* × *OoRr*). The predicted phenotypic ratio is:

9 red, round seed : 3 red, flat seed : 3 white, round seed : 1 white, flat seed

The expected F2 genotypic ratios are shown in the Punnett square below.

Parents	***OR***	***Or***	***oR***	***or***
OR	*OORR*	*OORr*	*OoRR*	*OoRr*
Or	*OORr*	*OOrr*	*OoRr*	*Oorr*
oR	*OoRR*	*OoRr*	*ooRR*	*ooRr*
or	*OoRr*	*Oorr*	*ooRr*	*oorr*

However, when this cross was actually carried out, only orange, round and white, flat seeds were obtained, with a phenotypic ratio of 3 : 1. This phenotypic ratio is not in accordance with Mendel's Law of Independent Assortment because both genes are located on the same chromosome and are inherited together. They are shown to be linked genes.

Because the alleles are inherited together, there is no crossing over and so only two types of gametes are produced (*AB* and *ab*). In cases such as this, the correct Punnett square to use is seen in the Punnett square below.

Parents	***OR***	***or***
OR	*OORR*	*OoRr*
or	*OoRr*	*oorr*

Linkage and recombination

Crossing over is a normal event that results in genetic exchange between non-sister chromatids. It is presumed to occur in most germ line cells going through meiosis. The probability of at least one crossover event occurring somewhere on the chromosome is high because there is usually at least one chiasma (point of crossing over between chromosomes) for each homologous pair in meiosis. Crossing-over models currently explain the observed ratios of phenotypes for linked genes that do not show the typical ratios; however, empirical evidence is yet to conclusively support the notion of crossing over.

Figure 7.5.4 shows two hypothetical genes, A and B, with their pairs of alleles *A*, *a* and *B*, *b*.

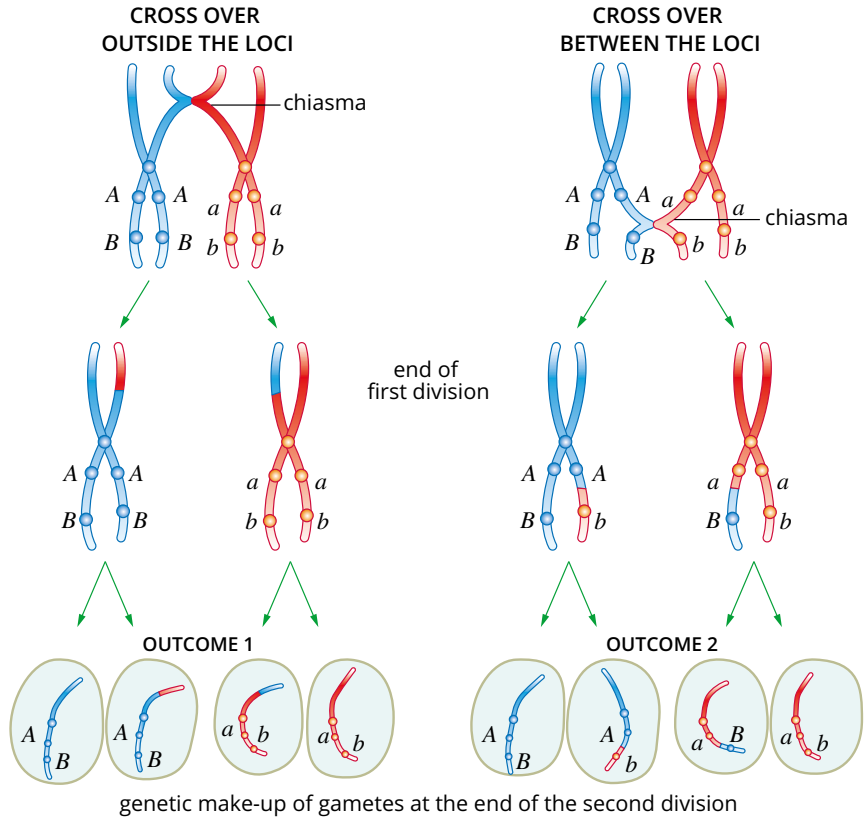


FIGURE 7.5.4 Illustration showing the consequences of crossing over. When two genes, A and B, are located on the same chromosome, crossing over may occur outside the loci (outcome 1) or between the two loci (outcome 2). The gametes produced in these two situations (outcome 1 and outcome 2) are very different.

The A and B loci are on the same chromosome. If a cross is initiated between *AA*, *BB* and *aa*, *bb* parents, the F1 of *Aa*, *Bb* (all heterozygote) individuals will be produced. There are two possible outcomes of meiosis in a cross of *Aa* and *Bb* heterozygotes.

- Outcome 1: crossing over does not occur between the A and B loci, but may occur elsewhere on the chromosomes. In this case:
  - only gametes of allelic combinations *AB* and *ab* are formed, and they are formed in equal frequencies
  - these gametes are referred to as being of the **parental type** because they are the gametes that the *AA*, *BB* and *aa*, *bb* parents of the F1 would have produced.

- Outcome 2: crossing over occurs between loci A and B. Gametes containing allelic combinations *AB*, *Ab*, *aB* and *ab* are observed in equal frequency. In this case:
  - some parental type gametes (*AB* and *ab*) are formed
  - **recombinant gametes**, *Ab* and *aB* are also formed.

The modelling assumes that if the A and B loci are very close together, the probability of a crossover event occurring in-between the two loci (outcome 2) is very low.

If genes are close together, there will be fewer recombinant gametes and more parental gametes produced (outcome 1). The closer the two genes are together, the rarer the recombinant gametes will be.

If genes are so far apart (on the same chromosome) that close to 50% of the gametes are recombinants, then independent assortment is observed. If the percentage of recombinant gametes is less than 50%, the two genes are considered to be linked (Figure 7.5.5).

Recombinant gametes carry a combination of alleles not observed in the parents.

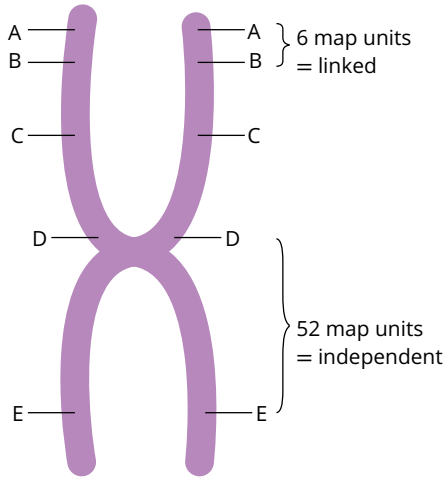


FIGURE 7.5.5 Genes located close together are considered linked. A map unit (MU) is not a physical distance, but a unit used to infer the percentage possibility of genes becoming separated during one event of crossing over. In the image above, genes A and B are 6 map units apart. This means they have a 6% chance of becoming separated during crossing over. Genes D and E have a 52% change of being separated during crossing over.

Linked traits: haemophilia and colour blindness

Pedigree analysis shows that two X-linked traits, red–green colour blindness and haemophilia A, tend to be inherited together (Figure 7.5.6a). This indicates that the genetic loci for these two genes are close and inheritance is linked.

In family B, another X-linked trait, haemophilia B, was shown to assort independently from the colour-blindness trait (Figure 7.5.6b). In family B, traits found in one individual (I-1) were inherited separately in subsequent generations (III-2, who inherited haemophilia B, and III-3, who inherited colour blindness). This shows that these two loci are farther apart, and therefore assort and are inherited independently.

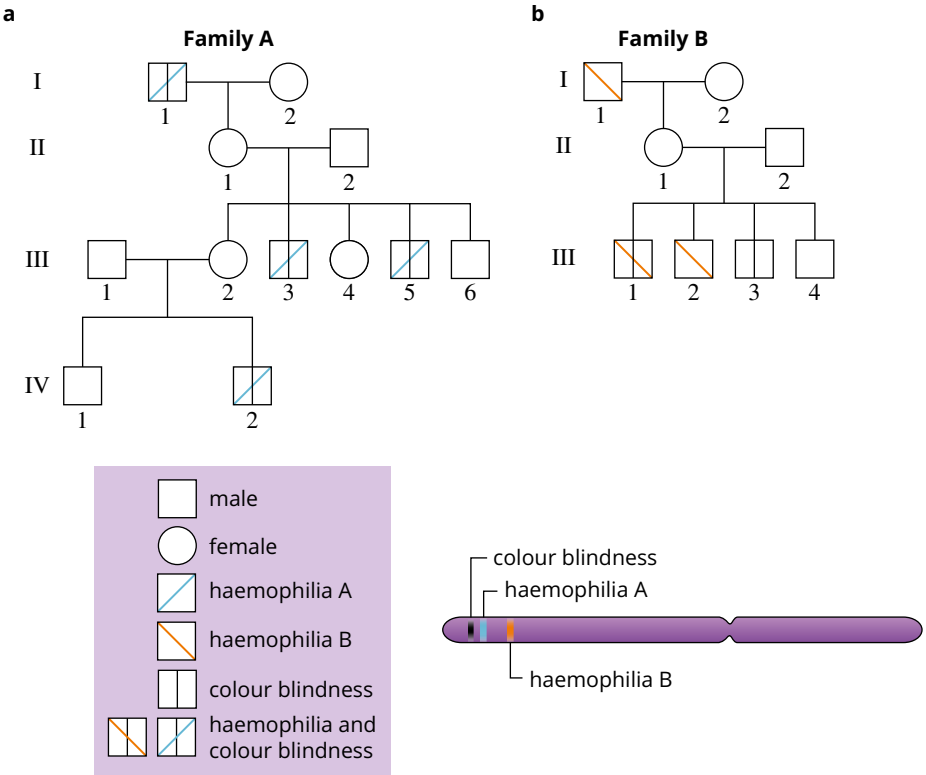


FIGURE 7.5.6 (a) Pedigree chart for Family A, affected by haemophilia A and colour blindness. (b) Pedigree chart for Family B, affected by haemophilia B and colour blindness. Alleles for these three traits are all on the X chromosome. The distance between the loci determines how likely they are to be inherited together. Colour blindness and haemophilia B are farther apart on the chromosome, so they are less likely to be inherited together than colour blindness and haemophilia A.

Recombination and distance between linked genes

By measuring the percentage of recombinant gametes produced by an F1 heterozygote when genes are linked, the distance between the two genes is estimated. The farther apart two genes are on the chromosome, the more frequently crossing over may occur and the higher the observed percentage of recombination. By repeating such measurements for different pairs of genes, the position of any identifiable gene on a particular chromosome can be found. This process is called **gene mapping**.

Molecular techniques and genetic markers play an important role in this area. Linkage relationships are becoming increasingly important in providing information about disorder inheritance. A marker locus closely linked with the gene that causes a disorder in a child, can be extremely useful in determining the genotype of a parent who has the disorder.

Making use of gene linkage

An important consequence of linkage is that very different traits can be inherited together. This can be extremely important in terms of understanding human health, and plant and animal breeding.

Information about one locus provides us with the likely genotype at the other locus for linked genes. A genetic marker is a sequence of DNA with a known location on a chromosome. If a gene of interest is closely linked to a genetic marker, then this can be used to determine if someone carries a mutation (a change in DNA sequence). The closer two loci are, the greater the linkage and the more precisely a genotype can be used at one locus to predict the other.

7.5 Review

SUMMARY

- A dihybrid cross is a cross between two individuals that carry alleles for different traits at two genetic loci.
- A phenotypic ratio approximating 9 : 3 : 3 : 1 will be observed in the F2 generation of a dihybrid cross if the two traits are each separately controlled by a single autosomal gene with two alleles, where for each trait one phenotype is dominant and the genes assort independently.
- Independent assortment occurs because the segregation of one pair of homologous chromosomes (and the alleles they carry) in meiosis does not influence the segregation of other homologous pairs of chromosomes.
- Linkage is the tendency for two or more genes located on the same chromosome to be inherited together.
- Genes are linked when the percentage of recombinant gametes falls below 50%.
- Recombinant gametes carry a combination of alleles not observed in the parents.
- The percentage of recombinant progeny can be used to estimate the distance between two genes on chromosomes.

KEY QUESTIONS

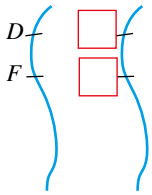
Retrieval

1 Recall Mendel's second law and what it states.
2 Define gene mapping.
3 Define linked gene.
4 Describe what the probability of a crossover between two gene loci depends on.

Comprehension

5 Determine the possible gamete genotypes as an outcome of meiosis in a heterozygote individual with the genotype *AaBb*, without linkage or crossing over.

6 Sheep blowfly chromosome 5 carries genes for resistance to the insecticide dieldrin (gene *D*). The same chromosome carries a gene called furrowed eyes (*F*).



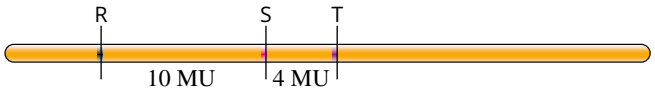
a Document the allele symbols for a fly that is heterozygous at both loci.

b Derive what combinations of alleles will be present in gametes if crossing over does occur.

c Construct a Punnett square for a cross between the fly shown above (genotype *DdFf*) (after recombination has occurred in meiosis) and a homozygous recessive fly (*ddff*).

d If dieldrin resistance is a dominant trait and furrowed eye is recessive, determine what proportion of the offspring with normal (wild type) eyes are resistant to the chemical dieldrin.

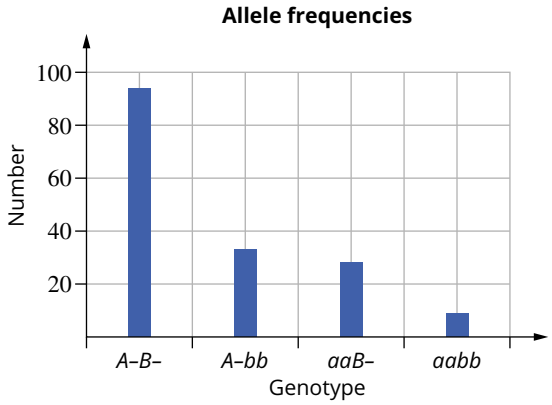
7 The diagram represents a linkage group on a chromosome from a common crop plant.



Explain whether you would expect the greatest percentage of recombination to occur between RS, ST or RT.

Analysis

8 From the histogram below, infer information about the genotypes of the parental population if the data represents the F1 generation. Dashes may represent more than one allele (for example, *B* or *b*).



9 A domestic fruit plant has phenotypes of tall and dwarf, and variation in fruit texture of smooth and furry. The tall phenotype is dominant to the dwarf, and the smooth fruit is dominant to the furry. The fruit grower is trying to establish a pure-breeding stock of tall plants with smooth fruit. To identify the homozygous specimens in her crop, she crosses dwarf, furry plants with tall, smooth ones. The results of one cross are set out in a table.

Fruit plant cross results

Phenotype	Frequency
tall plant, smooth fruit	88
tall plant, furry fruit	10
dwarf plant, smooth fruit	8
dwarf plant, furry fruit	94

The fruit grower was surprised at the proportions of the different offspring, as she was expecting a 1 : 1 : 1 : 1 ratio.

a Account for the actual results.

b Explain in what circumstances the expected 1 : 1 : 1 : 1 ratio might have been achieved.

c In terms of the fruit grower's aim in conducting this cross, reflect on what valuable information has been gained from the results.

Chapter review

07

KEY TERMS

aneuploidy
block mutation
carrier
co-dominance
continuous variation
cross
deletion mutation
dihybrid cross
discrete variation
dominance
dominant phenotype
duplication mutation
F1 generation
F2 generation
gene mapping
genotype
hemizygous
heterozygote
homozygote
hybrid
incomplete dominance
insertion mutation
inversion mutation
karyotype
Law of Independent Assortment
linkage
monogenic
monohybrid cross
non-disjunction
parental generation
parental type
pedigree analysis
phenotype
polygenic
polygenic inheritance
recessive phenotype
reciprocal cross
recombinant gamete
sex-limited inheritance
sex-linked inheritance
test cross
translocation mutation
true-breeding
wild type
X-linked
Y-linked

KEY QUESTIONS

Retrieval

1 Recall how many non-homologous pairs of chromosomes you would expect to find in the cells of most:
- **a** human females
- **b** human males

2 A test cross that considers two gene loci positioned on different chromosomes is expected to yield a phenotypic ratio of:
- **A** 3 : 1
- **B** 1 : 1
- **C** 9 : 3 : 3 : 1
- **D** 1 : 1 : 1 : 1

3 Recall the name given to the point at which two chromosomes cross during meiosis.
- **A** histone
- **B** chiasma
- **C** homologue
- **D** centromere

4 Categorise data that can take any value within a range.
- **A** outlier
- **B** discrete
- **C** qualitative
- **D** continuous

5 Recall the general type of traits that show continuous variation.

6 Recall the name given to an individual that has two different alleles for the one gene.

Comprehension

7 Summarise the variations seen in eukaryotic chromosome structure.

8 Clarify why the genotype and phenotype of an individual do not always match.

9 Recognise what a nucleosome consists of.
- **A** DNA and histones
- **B** RNA and histones
- **C** DNA and chromatid
- **D** Chromatid and nucleotides

10 Describe karyotyping and one application of its use.

11 Explain the difference between the following terms:
- **a** homogametic and heterogametic
- **b** heterozygote and homozygote

12 For each of the conditions listed below, determine the number of chromosomes in a karyotype of an individual with the condition.
- **a** Down syndrome
- **b** Turner syndrome
- **c** Klinefelter syndrome

13 *P* and *p* are alleles of a particular autosomal gene. Identify the allelic combinations and name them appropriately. Give the dominant or recessive phenotype.

14 Mutations affect phenotype. Explain the likelihood of the next generation inheriting these changed phenotypes.

15 Identify which of the following genotypes shows alleles for a heterozygous trait.

A AA
B Bb
C CD
D Cd

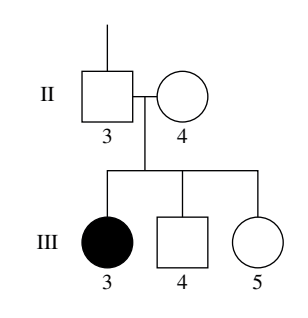
16 If a woman is a carrier for a sex-linked recessive disease, only a son can suffer from the disease if the father does not have the disease. In vitro fertilisation enables the sex of the embryo to be determined very early in development. It is therefore possible to implant only female embryos into the woman's uterus so that the disease will not occur in her children. Explain whether this approach means that the disease will not occur in subsequent generations of the family.

17 Select which of the following alternatives is correct. Alleles for two different genes positioned at opposite, extreme ends of a particular chromosome can be expected to:

A be linked
B be inherited together
C assort independently
D separate at the centromere at anaphase

Analysis

18 The figure shows part of a pedigree chart.



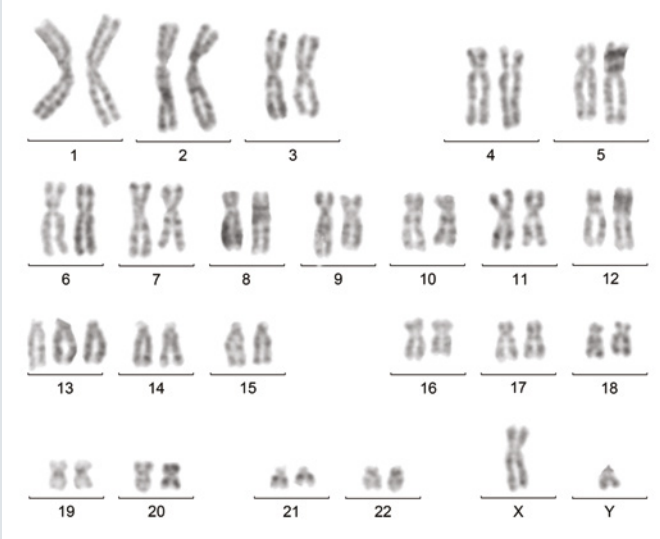
a Determine which of the following best describes the trait shown by the shaded individual (III-3).

A dominant
B sex-linked
C recessive
D co-dominant

b Deduce the genotypes of the parents of III-3. Choose from options A–D and draw a Punnett square to show your reasoning.

A *BB*, *BB*
B *Bb*, *Bb*
C X^BX^B, X^BY
D X^BX^B, X^bY

19 The figure below shows a human karyotype.



a Determine whether this individual is male or female.

b Interpret the karyotype to determine whether there is any evidence of aneuploidy in this person.

20 Mice from a pure-breeding line of black coat colour are crossed with mice of a pure-breeding strain of white coat colour. The F1 offspring all have black coats. F1 progeny are crossed to produce F2, in which 64 mice have black coats and 20 have white coats. Reciprocal crosses yielded similar results.

a Conclude a genetic hypothesis to explain these data. Your hypothesis should specify which phenotype is dominant and how many genes are involved. Give the genotypes of the parental lines and the F1 and F2 offspring.

b Deduce the inheritance pattern for black and white coat colour in mice.

c **i** State how many genes are involved.
ii Explain which data supports your claim.

d Use appropriate notation to identify the respective parental genotypes. Carry out the crosses to show how the results of the F1 and F2 generations were achieved.

21 The shape of a human earlobe is determined by a single autosomal gene. Free lobe is dominant to attached lobe.

a Symbolise the alleles for this gene appropriately.

b Determine how many genotypes are possible with respect to these alleles.

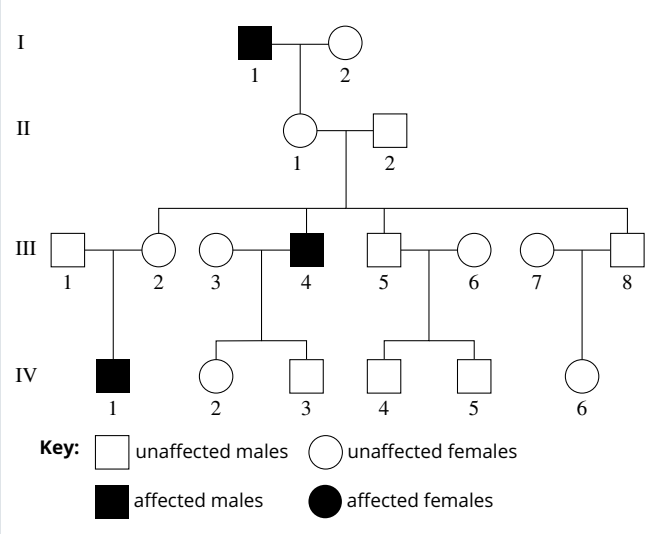
c A homozygous man with free lobes married a heterozygous woman. Document the genotypes and phenotypes possible in their children.

d Calculate if it is possible for two people with free lobes to have a child with attached lobes.

e Two parents who are both heterozygous for earlobe shape have a child. Calculate the probability that the child has attached lobes. Write your answer as both a percentage and a ratio.

22 Using the key terms that relate to different sorts of crosses (e.g. test cross, monohybrid cross, dihybrid cross) organise a poster that distinguishes between the different crosses.

23 The pedigree in the following diagram illustrates the pattern of inheritance for a particular characteristic.



a **i** State the mode of inheritance for this characteristic.

ii Describe the evidence that suggests this mode of inheritance.

b Use appropriate notation to assign genotypes to individuals I-1, II-1, III-2, III-4, III-5 and IV-2.

c Predict possible genotypes for individual III-7. Explain your reasoning.

d State the possible genotypes and phenotypes in the children fathered by individual IV-1 if his partner were to have no family history of the trait.

24 Consider the inheritance of flower colour and pollen grain shape in sweet pea. Purple flower colour (P) is dominant to red flower colour (p), and long pollen grain shape (L) is dominant to the round pollen grain shape (l). When homozygous purple-flowered long pollen grain plants are crossed with homozygous red-flowered round pollen grain plants, the offspring are all purple-flowered with long pollen grains.

a Write down the genotype of the F1 generation.

b Use a Punnett square to illustrate the expected ratio of genotypes and phenotypes when the F1 offspring are test crossed to the red-flowered round pollen grain strain, considering the principle of independent assortment.

c The results of the test cross do not reveal the expected ratio of phenotypes for these two characteristics. The observed number of phenotypes are set out in the following table.

Observed number of phenotypes in sweet pea test cross

Phenotype	Number
purple flower, long pollen grain	190
purple flower, round pollen grain	29
red flower, long pollen grain	24
red flower, round pollen grain	178

i Account for the observed ratio.

ii Name the process that has occurred to produce the purple-flowered round pollen grain and red-flowered long pollen grain offspring.

iii Use the following formula to calculate the map units between the linked genes:

$$\text{map units} = \frac{\text{number of recombinants} \times 100}{\text{total number of progeny}}$$

25 Shrunken endosperm in corn kernels is a recessive characteristic. Plump endosperm is dominant. Coloured endosperm is determined by a single gene. Two homozygous plants are crossed. The phenotype of the kernels in the F1 is plump and coloured. F1 plants are test crossed and the resultant offspring have the phenotypes: 110 shrunken and colourless, 100 plump and coloured, 6 shrunken and coloured, and 4 plump and colourless.

a Determine the genotypes and phenotypes of the original parents.

b Show the genotypes of zygotes and gametes at each stage of the cross.

c Explain why the test cross offspring depart from a 1 : 1 : 1 : 1 ratio.

26 A pure-breeding strain of blowfly with small orange eyes is crossed to wild type flies with large red eyes. The following numbers of offspring are observed in each phenotypic class: 91 wild type, 12 large orange eyes, 9 small red eyes, 103 small orange eyes.

- **a** Explain whether the wild type parents were all pure-breeding.
- **b** Justify which characteristics are dominant.
- **c** State what type of cross this is.
- **d** Show the genotypes of the parents and their offspring and provide a genetic hypothesis to explain the results.

Knowledge utilisation

27 Create a flow diagram that enables the identification of the pattern of inheritance equivalent to Figure 7.4.14 (page 363). Start with the question: Is the condition observed in both males and females?

28 Generate a test for the following hypothesis: X-linked recessive traits cannot be differentiated from autosomal dominant traits using pedigree charts.

29 Human eye colour is an inherited trait that has been significantly studied in recent times and our understanding is continually growing. Currently, it is thought that two genes, classified as *OCA2* and *HER2*, are the major contributors. The *OCA2* gene produces a protein that is involved in producing and storing melanin in the iris. Melanin has a brown colour. The *HER2* gene regulates the activity of the *OCA2* gene. When these genes do not work properly, a blue eye colour is the result. It is also known that several other genes influence the activity of *OCA2* and *HER2* by decreasing their activity. In the last few years, a number of *OCA2* and *HER2* single-nucleotide polymorphisms (a genetic variation between members of the same species, affecting a single nucleotide) have been discovered and observed to have varying effects on how much their activity is lowered. It has been stated you have either brown or non-brown eyes, and eye colour is understood as being on a spectrum from brown to blue.

Draw conclusions about the role of Mendelian genetics and mutations in the inheritance of eye colour. (Extended response: 300–350 words)

CHAPTER 08

Biotechnology

Biotechnology is the use of living things to manufacture products to improve the quality of our lives. Humans have used microbes to make products for us for several thousand years, for example, yeasts in bread, wine and cheese. Now society looks to modern biotechnology to fight diseases, reduce our environmental footprint (for example, cleaning up oil spills), provide food and clean energy and develop more efficient industrial processes. As our understanding of DNA and genetics advances, we are able to develop techniques to manipulate DNA. Research in biotechnology (and other related areas, such as medicine and biology) continues to advance rapidly because of our ability to manipulate an organism's genetic material.

In this chapter, you will investigate genetic modification of organisms using recombinant DNA techniques. You will gain an understanding of the various sequencing methods and the ways we can generate and store the incredible amount of data that result from sequencing techniques. You will also learn about DNA profiling and some of the current uses of genetic biotechnology. You will also consider some of the social, ethical and legal issues associated with biotechnology.

Syllabus subject matter

Topic 1 • DNA, genes and the continuity of life

BIOTECHNOLOGY

- describe the process of making recombinant DNA
  - isolation of DNA, cutting of DNA (restriction enzymes)
  - insertion of DNA fragment (plasmid vector)
  - joining of DNA (DNA ligase)
  - amplification of recombinant DNA (bacterial transformation)
- recognise the applications of DNA sequencing to map species' genomes and DNA profiling to identify unique genetic information
- explain the purpose of polymerase chain reaction (PCR) and gel electrophoresis
- appraise data from an outcome of a current genetic biotechnology technique to determine its success rate.

SCIENCE AS A HUMAN ENDEAVOUR

Genetically modified organisms: Transgenic organisms have potential for advancement in agriculture and pharmaceuticals.

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8.1 Biotechnology and genetic engineering

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- understand the problems associated with organising and storing the vast amount of data generated from DNA sequencing
- recognise the uses of DNA sequencing and editing and its potential for our future and the rest of life on Earth
- recognise current biotechnology techniques such as CRISPR and evaluate their impact on our lives.

The molecular tools and techniques used to manipulate DNA molecules for particular purposes are discussed in this section. Organising and making sense of the vast array of data that this produces has implications for all of us. You need to know what data is being collected in DNA profiling, how it can be used and how it can affect your life if it is misused. Choices about genetically modified food, medical tests and interventions will affect everyone. You need to be able make decisions based on an understanding of basic principles and their limitations.

BIOINFORMATICS

Bioinformatics uses computer programs and models (algorithms) to organise the results measured when analysing biological **macromolecules**, such as DNA and protein sequences. Due to the large amount of information produced from analysing DNA and protein sequences, computer programs and models are essential for comparative studies. The first nucleotide sequence of a virus with 5386 bases was published in 1977. Since then, public access to DNA sequences has grown 100 million-fold. Now agricultural scientists, medical researchers, ecologists, biotechnologists all need to know the exact set of bases, signals and control mechanisms for the genes they are investigating.

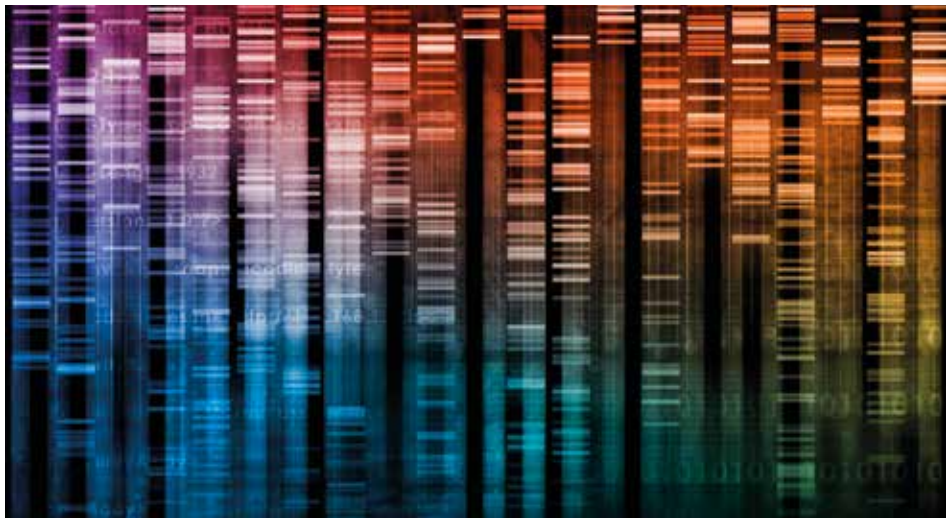

FIGURE 8.1.1 DNA sequencing tracks for computer analysis.

Each of the two main international centres for bioscience data—the US National Centre for Biotechnology Information and the EMBL European Bioinformatics Institute—now manages almost 10 petabytes of data (10 million gigabytes). As we enter the era of personal genomics, there is every reason to expect this rate of growth not only to continue but to accelerate.

DNA sequencing generates a large amount of data and computer analysis is the only way to handle the quantity and variety of information (Figure 8.1.1). Previously this was done by hand, with no real way of using and storing the vast amount of information. It is estimated at the moment that the databases containing the gene sequences of organisms doubles in size every 15 months. **Basic Local Alignment Search Tool (BLAST)** is software that has an algorithm for finding and comparing biological sequence information, such as the amino acid sequences of proteins or the sequence in polynucleotide chains of DNA. This can help determine species genome maps, evolutionary relationships (Figure 8.1.2), gene regulation sites and sequences of proteins related to disease.

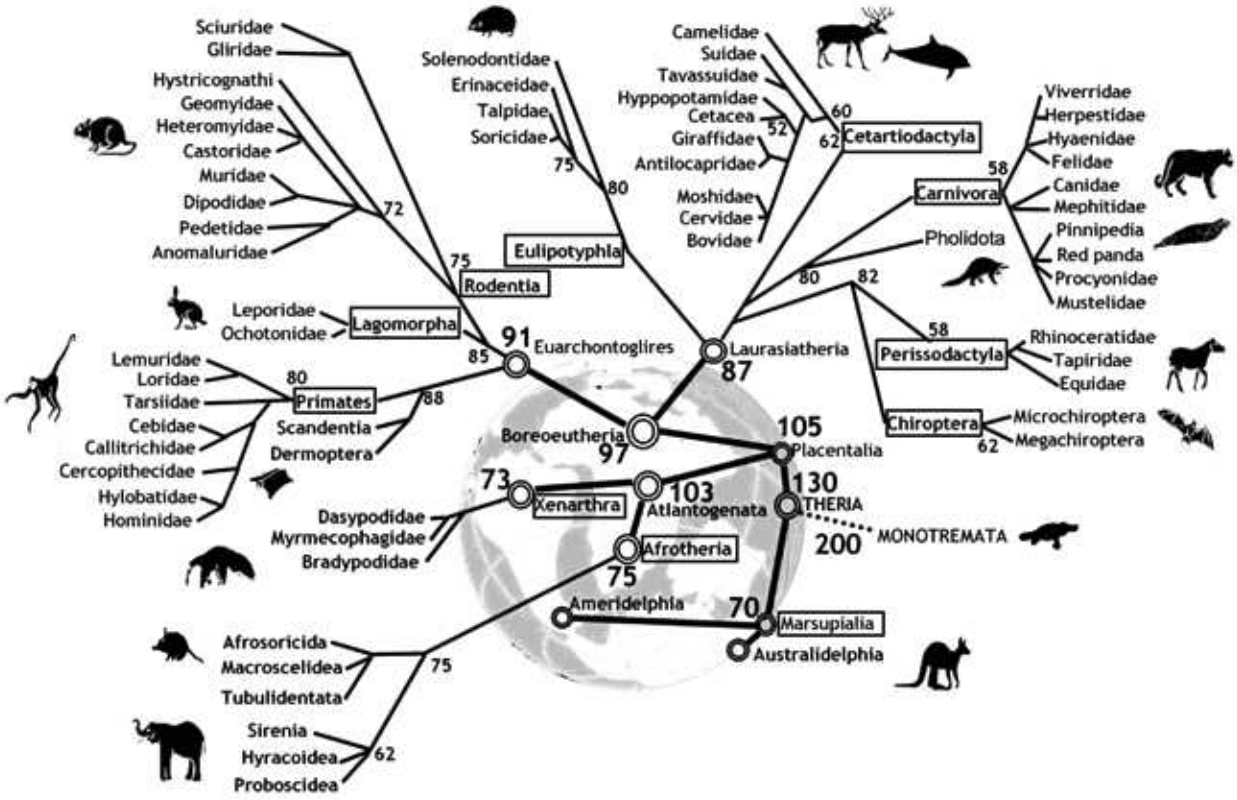

FIGURE 8.1.2 Bioinformatics is used to help us understand the genetic relationships between species, such as the relationship between mammal species seen in this evolutionary tree.

Human Genome Project

The **Human Genome Project** was an international scientific research project to determine the sequence of nucleotide base pairs that make up human DNA. It was started in 1990 and finished in 2003, two years ahead of schedule. All of the functional genes (genes that encode functional proteins) of the human genome were identified and mapped. Geneticists are now identifying the role of each of these genes in biological processes, including insulin regulation in diabetes, genetic **predisposition** to heart disease and the adaptive immune system response to pathogens and vaccines. More genes and their biological functions are being identified each year. The main focus of the research is now a better understanding of the action and interaction of genes.

Doctors may recommend genetic screening to identify genetic abnormalities and disorders, while commercial companies can sequence your DNA to give clues to your ancestry or develop a health insurance policy considering your predisposition to diseases or disorders. But there are social, biological and ethical implication for storing very personal information in databases. Figure 8.1.3 summarises these issues.

social implications

- data mining where details are gathered from different databases to build up a profile of an individual without their consent
- privacy and confidentiality
- bias towards individuals (e.g. for insurance purposes, or job prospects)
- social equality/inequality
- unreal expectations based on genetic potential
- social stigma associated with certain marker genes that may never result in disease
- unknowingly finding out about a disease in the family from another family member's profile

biological implications

- changing the course of evolution-altered inheritance
- ability to correct mutations
- accuracy (possible false positives and false negatives)

ethical implications

- commercial exploitation of human DNA sequences – should they be copyright?
- dehumanising individuals, machines making decisions about our future
- informed consent for DNA sampling
- security of stored genetic data
- legal access to genetic data
- reliability of DNA evidence as proof of guilt or innocence in criminal matters
- misinterpretation of statistics linking marker genes to disease
- couples requesting screening to make reproductive decisions
- bioweapons
- sequencing babies at birth

FIGURE 8.1.3 Summary of issues arising from storing personal information on a database.

Applications of bioinformatics

There are a range of ways that bioinformatics can be applied to improve human health and wellbeing, and to improve industry.

Molecular medicine

Molecular medicine involves the use of bioinformatics and physical, chemical, biological and medical techniques to describe molecular structures and mechanisms, identify fundamental molecular and genetic errors of disease, and develop molecular ways to manage them.

Molecular medicine has great potential. It could allow the identification of the exact drug target for medicines, providing effective treatment. Additionally, it is anticipated that knowing the drug target will result in treating many current diseases rather than just managing their symptoms. This could reduce costs, minimise the time associated with drug discovery and allow for the exploration of the causes of disease at the gene level.

Personalised treatment

Doctors could have the genetic profile of a patient and use that to prescribe a drug and dosage for that patient from the start of treatment. This information could be used alongside the physical health profile of the patient and lifestyle factors to potentially prescribe a more accurate dosage with less error. Researchers hope personalised treatment through genetic profiles will decrease the number and severity of side effects of drugs and minimise the time needed for treatment. However, the complexity of genetics makes this a challenging task.

Preventative medicine

Preventative medicine uses biotechnology to find methods to prevent disease before treatment is necessary. Where genetic tests can be developed to identify genetic disorders, preventative steps can be taken before the disease can take hold. For example, doctors may soon use **gene therapy** to treat, cure or prevent a disease by altering the patient's gene expression. After decades of research, the first few gene therapy treatments became available in 2017. These included gene therapy treatments for a form of leukemia and sickle cell anaemia. Preventative medicine may also be as simple as a change of diet to avoid triggering a whole range of symptoms once the predisposition to the disease has been identified. Current gene therapy treatments can cost as much as $1 000 000 per treatment.

Microbial genome analysis

By knowing the genome of microbes, scientists can isolate genes that help microbes survive extreme conditions. For example, the DNA sequence of *Caulobacter crescentus* (Figure 8.1.4), a microbe involved in sewage treatment, was determined. This enabled scientists to investigate the cell cycle in detail due to its unusual cell division and to isolate the genes that may help other organisms survive in adverse conditions.

Another example is the analysis of microbes that use carbon dioxide (CO_2) as their sole carbon source. Scientists researching alternative energy sources are investigating *Chlorobium tepidum*, a bacterium that generates energy from light and may be the key to one of the most efficient energy sources ever discovered. Other useful biotechnology microbes include *Archaeoglobus fulgidus* and *Thermotoga maritima*, which survive in temperatures above boiling and can be used for extreme industrial processes, and *Corynebacterium glutamicum*, which is used to produce the amino acid lysine. Lysine is used in feed concentrates as an alternative to meat or soybeans, and can be used when the pastures can no longer support livestock.

Mycoplasma genitalium has one of the smallest genomes at 0.58 mega base pairs (580 000 base pairs), while *Mycobacterium tuberculosis* has a genome 4.41 mega base pairs (4 410 000 base pairs) long.

Scientists looking at antibiotic resistance have discovered a **virulence** region called 'pathogenicity island' in *Enterococcus faecalis* (a leading cause of infection in hospital patients) and manipulation of this could be used to control its spread in hospital wards. Forensic analysis scientists were able to distinguish between strains of *Bacillus anthracis*, which was used in a terrorist attack in 2001 in America. **Bioweapon** scientists have created a synthetic polio virus from instructions and materials readily available on the internet. Evolutionary studies are underway to fine tune our understanding of the relatedness between different species, starting with bacteria.

FIGURE 8.1.4 *Caulobacter crescentus* has several genes and clusters of genes that are essential for survival in habitats with poor nutrients, allowing the bacterium to respond to a wide range of environmental fluctuations.

Human microbiome

Human microbiome refers to the viruses, fungi and bacteria that live in or on us. Recent studies of human microbiomes has shown links between microbiomes and some disorders. For example, children with **autism** tend to have less diverse gut bacteria than those who are not affected by the disorder. Specifically, *Prevotella* species were rarely found among samples from children with autism, especially *Prevotella copri* (Figure 8.1.5), which helps the breakdown of protein and carbohydrate foods. However, it is not clear what the relationship is between autism and microbiome. Projects such as MetaHIT in Europe, Human Microbiome Project in the USA, and Integrated Microbial Genomes are investigating this link.

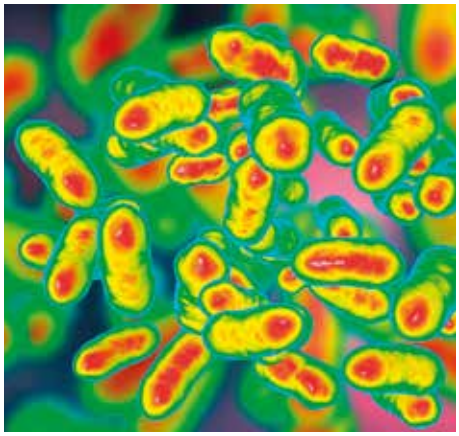

FIGURE 8.1.5 3D illustration of *Prevotella* bacteria.

Agriculture

Bioinformatics can be used to help produce a greater variety of crops, with more disease and insect resistance. Using bioinformatics to transfer genes from one organism to another creates a **genetically modified organism (GMO)**. This is explained in more detail in the next section. Genes from *Bacillus thuringiensis* have been successfully transferred to cotton, maize and potatoes, reducing the amount of pesticides needed for these crops. To increase nutritional quality, scientists have transferred genes into rice to increase the concentration of vitamin A, iron and other micronutrients. Flavr Savr tomatoes have a gene from yeast that enables them to stay on the vine longer to ripen and gives them a longer shelf life. Crops that can grow in poor soils, like cereal varieties, have been developed to have greater tolerance for soil alkalinity, free aluminium and iron toxicities so they succeed in poorer-growing areas and are drought-resistant. Livestock will improve in production (increasing the muscle mass during growth or accelerating growth, milk composition) and health (decreased disease susceptibility such as to mastitis and mad cow disease, and strengthening the immune system).

Current technology: CRISPR

An example of molecular medicine is the development of a new technique using DNA sequences from bacteria called **CRISPR**, which can cut DNA at specific locations. CRISPR stands for 'clustered regularly interspaced short **palindromic** repeat', which means short segments of DNA from bacteria that have survived being attacked by a virus due to its function.

The CRISPR system is a **gene editing** technique originally found as part of a bacterium's defence system. The natural intracellular system, the CRISPR DNA sequence, derives sequences from viruses that have previously attacked the bacterium. The CRISPR sequence then synthesises CRISPR RNA and **Cas proteins**. The CRISPR RNA is able to accurately recognise specific nucleotide sequences from previous virus attacks and guides the Cas proteins to the virus. The Cas protein then destroys the genetic material of the virus.

CRISPR can edit genes by combining a DNA-cutting enzyme with a molecular guide that can be programmed to tell the enzyme precisely where to cut. Scientists are learning to use synthetic **guide RNA (gRNA)** with Cas proteins to cut eukaryotic DNA at a desired location (Figure 8.1.6). The synthetic gRNA is artificially created with a desired short length of genetic code. It guides the CRISPR system to the target location where scientists want to edit genes.

Clinical trials in humans have already started with cancer genes and editing genes in embryos.

The current understanding of the Cas9–gRNA system is limited. The success rate for the number of genes or cells edited is not well known. Also, recent developments have shown that not every position in the gRNA needs to match the target DNA, resulting in off-target (unintended) sites being edited. Currently, research results regarding CRISPR efficiency are elusive. There is a large range of success rates for three different genes (13% to 43%), and success rate in the growth of transgenic mice embryos ranges from 2% to 88%.

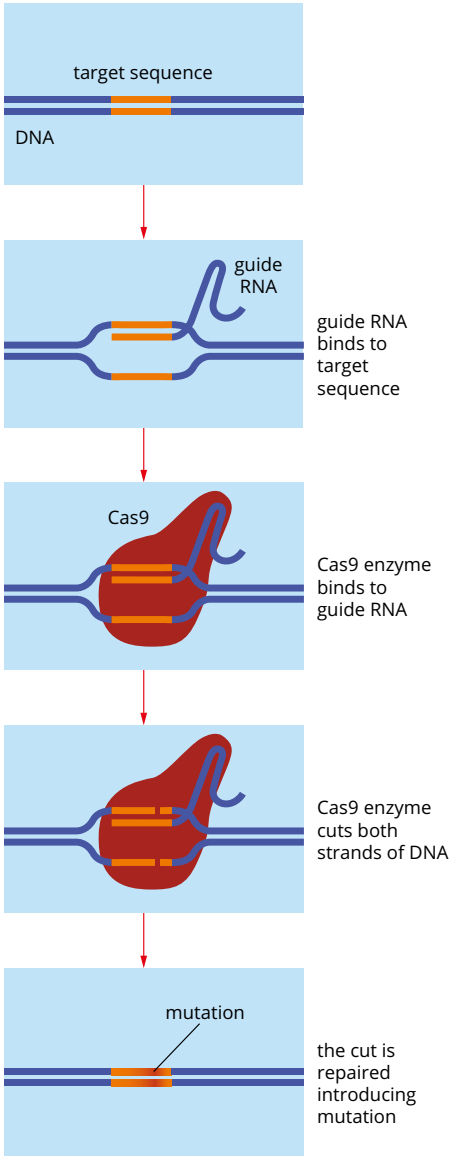

FIGURE 8.1.6 Diagram of the CRISPR–Cas9 editing tool.

The use of CRISPR to edit the genome in **germline** cells (eggs or sperm) raises bioethical concerns and at the moment, **patent rights** to the technique are being contested with several companies. Laboratories have started work on human embryos, deleting harmful genes, but with mixed reactions. If gene modification occurs in embryos, not all the cells may carry the edited copy, but any changes made to eggs or sperm will affect all the cells in the embryo that develop once the egg is fertilised. Any changes made will be passed on from generation to generation and at the moment the use of reproductive cells for CRISPR studies is illegal in most countries.

Most of the gRNA (guide sequences) being tested at the moment are only around 20 bases long and such a short sequence is likely to exist somewhere else in the genome unrelated to the target site. This means crucial areas of other genes may be accidentally affected as well. It will be many years before CRISPR will be routinely used in humans.

GENETICALLY MODIFIED AND TRANSGENIC ORGANISMS

Humans have used selective breeding to produce animals and plants with more useful or more attractive characteristics for tens of thousands of years. We chose those animals or plants that expressed the characteristics we wanted to conserve and selectively bred them together, hoping that their offspring would show even more of these characteristics.

In the past, selective breeding could only utilise characteristics that already existed in the genetic pool of a species. We now have the knowledge and skills to use recombinant DNA techniques to transfer genes from one species to another to produce organisms with DNA combinations never seen before (Figure 8.1.7). This may lead to many benefits, but it also raises questions about whether the impacts of this manipulation are socially or ethically acceptable.

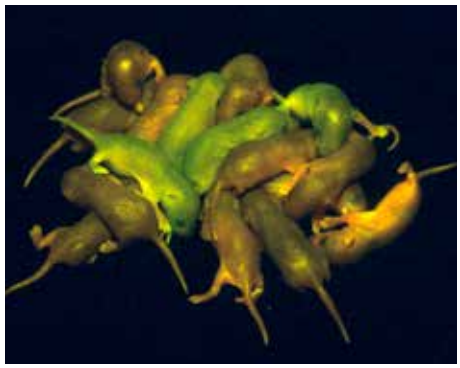

FIGURE 8.1.7 Fluorescent newborn mice are a result of a protein from fireflies being successfully transferred into the sperm of their father.

Over the last few decades, techniques have been developed that allow for the alteration of an organism's genome and for the transfer of genes from one organism to another (**genetic modification**). Because the DNA code is universal, almost any gene transferred from one organism to another will express the protein that it expressed in the original organism. This means that a desirable characteristic seen in one animal or plant can potentially be transferred to another organism lacking this characteristic. One example is the DNA code for a fluorescent protein from fireflies being transferred into the DNA of mouse sperm. The protein is not only synthesised in the newborn's cells, it is folded in to its unique 3D shape and becomes fully functional, capable of emitting light.

Genetically modified organisms

An organism that has had its genome altered in this way is considered to be a GMO. GMOs may have had their genome modified by directed mutation (mutagenesis) or by newer technologies called gene editing.

One of the first commercial uses of a GMO was the production of human insulin. Insulin had been extracted from the pancreas of pigs or cows up until the 1980s and often caused allergic reactions because animal proteins contaminated the hormone solution. Scientists were keen to insert the human gene for insulin into a bacterial template to allow large-scale production of what is now known as GMO insulin or humulin (Figure 8.1.8).

FIGURE 8.1.8 Genetically engineered insulin, humulin, is preferred over animal-based products.

The GMO insulin is identical to insulin produced by the human pancreas. The supply is unlimited, does not depend on animal sources, is cheaper and has no adverse reactions. Some patients have reported side effects and pharmaceutical companies are constantly trying to improve the efficiency of the drugs they produce.

Transgenic animals

Transgenic organisms are GMOs that have had a gene from another organism inserted into its genome. The gene that came from another organism is called a **transgene**.

Transgenic animals have been used in scientific research (Figure 8.1.9), medicine, pharmaceutical production and agriculture. In research, mice, rats and rabbits are used as living models of biological processes in healthy and diseased states. Scientists can study disease progression and potential treatments. For example, scientists studying motor neuron disease have identified a mutation in the gene for the enzyme superoxide dismutase 1 (SOD1) that is associated with some inherited cases of this disease. To study the disease in an animal model, scientists produced a transgenic mouse expressing the SOD1 enzyme mutation to establish links between the mutation and disease symptoms and investigate treatments.

Scientists may also produce knock-out mouse models in which gene technology is used to disable (or knock out) the expression of a particular gene. The phenotype (appearance, behaviour and biological function) of the animal gives the first indications of the function of the protein encoded by the gene. Structural and regulatory genes are studied in this way.

In agriculture, transgenic sheep and cows are used for improved fertility, meat production, milk quality and yield, and wool quality and yield. The use of genetically modified farm animals has not expanded to the extent it has for plants, perhaps because of detrimental effects of some modifications in animals. For example, genes that promote growth may also cause altered skeletal growth, arthritis and heart and kidney problems.

To date, no genetically modified animals have been approved for human consumption in Australia. Transgenic fish (Figure 8.1.10) have been approved in the USA, but they are unlikely to be approved for sale in Australia in the near future. A gene from another salmon species, along with a promoter sequence from a fish called a pout, means that the transgenic salmon eat all through the year rather than just when the water temperature is warm. This increases the growth rates of these fish dramatically and means they are ready for harvest much sooner than non-modified salmon. The eggs of the genetically modified salmon are treated to create infertile adult fish (99% of the adults are reported to be sterile). This reduces the chances of them interbreeding with wild salmon if they escape from their pens. This will be the first genetically modified animal of any type to be cleared for human consumption in the USA.

Some farm animals are being used for the production of therapeutic proteins such as antibodies that are difficult to make in bacteria and cultured cells. This process has been referred to as 'pharming', combining the words 'farming' and 'pharmaceutical'. The products are released into blood or milk and can be readily extracted from there.

Spider silk protein (Figure 8.1.11) is an example of a potentially useful product made in transgenic goats. The gene for spider silk has been put into the genome of goats, along with regulatory genes, and is expressed in the goat's milk. Spider silk has extraordinary strength and flexibility. Potential applications include a biopolymer for artificial ligaments and tendons, bandages, biodegradable bottles and bulletproof clothing.

Genetically modified organisms (GMOs) are organisms that have had their genome altered in some way. If no new gene is introduced into the GMO, it is simply known as a GMO. A GMO that has had genes from another species inserted into its genome is also considered a transgenic organism.

FIGURE 8.1.9 This transgenic mouse carries the gene for green fluorescent protein (GFP). The *GFP* gene is linked to the gene being studied, and acts as a reporter gene for the expression and location of the protein of interest.

FIGURE 8.1.10 Transgenic salmon cleared for human consumption.

FIGURE 8.1.11 Genetic engineering is being used to produce spider silk in goat's milk.

Medical researchers are also exploring ways to modify genes in insect vectors of disease. For example, the gene for red fluorescent protein has been linked to a lethal gene to allow researchers to identify the genetically modified mosquitoes (Figure 8.1.12). These genetically modified insects are released into the wild where they transmit the lethal gene to their offspring to help control virus-carrying mosquito populations.

Researchers are also working on modifying pig embryos to make their organs suitable for transplantation into humans. The transplantation of organs from one organism to another is called **xenotransplantation**.

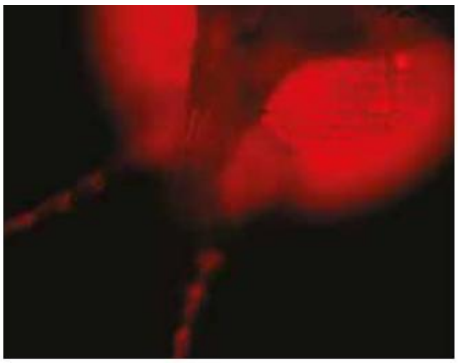

FIGURE 8.1.12 A genetically modified mosquito carrying both a lethal gene and the gene for red fluorescent protein.

Xenotransplantation is the transplantation of living cells, tissues or organs from one species to another.

Transgenic crops

Transgenic crops are used in agriculture to increase crop productivity, provide resistance to insect predation and prevent disease. Several genetically modified (GM) crops have been developed or are grown in Australia. For example, insect-resistant GM cotton has been grown since 1996 and herbicide-tolerant GM canola was approved for commercial production in Victoria in 2008. In Australia, the Office of the Gene Technology Regulator assesses all GM animals or plants before research, agricultural or commercial activities can occur.

Techniques for producing transgenic plants

Transferring a gene into plant cells can be limited by the presence of the cell wall. The introduction of foreign genes into plants is usually done with a biological vector. One method uses *Agrobacterium tumefaciens*, a soil bacterium that is able to naturally transfer a plasmid into plant cells (Figure 8.1.13). *Agrobacterium* normally causes crown gall disease, because it carries a plasmid with genes that cause the growth of a tumour. A recombinant plasmid (the vector), carrying a desired gene from a different species but lacking the tumour-inducing genes, is introduced into *Agrobacterium* cells. When the transformed *Agrobacterium* is cultured with plant cells, the recombinant plasmid is transferred into the plant cells. These transformed plant cells are then grown in tissue culture into new plants for transplanting into the field as a transgenic crop.

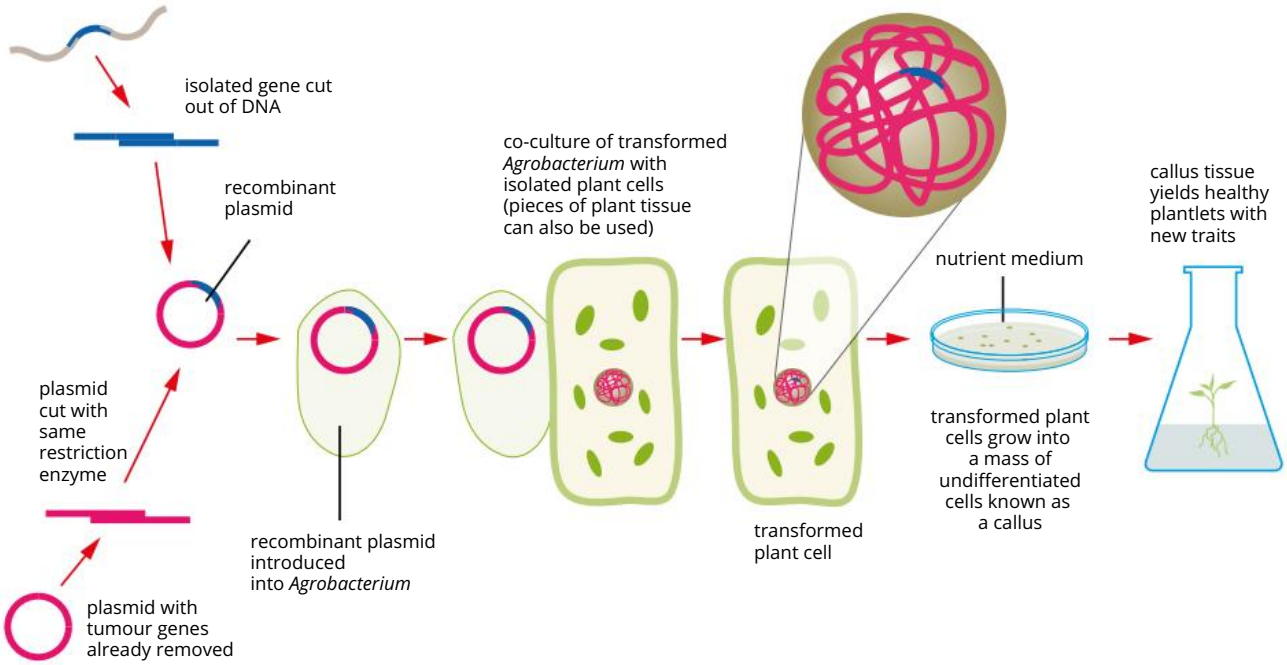

FIGURE 8.1.13 The use of *Agrobacterium tumefaciens* in gene cloning and the production of genetically modified and transgenic plants. Relative sizes of the plasmid, bacterium and plant cell are not to scale.

Salt-tolerant wheat

FIGURE 8.1.14 Australian scientists have produced wheat plants that can grow in salty soil.

Soil salinity is a major problem for Australian agriculture. A high level of sodium salts in the soil leads to osmotic water loss from roots and other tissues in which salt accumulates. Cells are stressed due to the altered ratios of sodium and potassium ions in cells. Salt-tolerant plants protect themselves from the effects of salinity by preventing sodium entry into cells, storing the salt in the vacuole or pumping the sodium out of the cells. Molecular biologists have found the genes that control these features of salt-tolerant plants.

To increase crop productivity, Australian scientists from the University of Adelaide introduced a gene from a salt-tolerant Australian native plant into wheat plants. This greatly improved the grain yield of wheat grown on salty soils without affecting grain yield in normal soil (Figure 8.1.14). The salt-tolerant gene codes for a protein that removes sodium from the leaves, allowing water to move normally from the roots to the leaves. This increases the geographical range that can be used for wheat production in Australia and other countries facing salinity problems, which is becoming increasingly important as the global population grows.

Bt cotton

Cotton is a plant that attracts many insect pests. To protect the cotton crops, they are sprayed with insecticides up to four times before the crop is harvested. This high use of insecticides impacts the populations of both harmful and beneficial insects and the animals that feed on them. Insecticides may also have an impact on human health. In addition, insecticides are expensive.

Bt cotton is a transgenic crop that has been modified to contain two genes from the soil bacterium *Bacillus thuringiensis*. Expression of these genes produces proteins in the cotton plant that kill the main caterpillar pest of cotton by disrupting its digestive system. In Australia, almost all cotton grown is Bt cotton and this has reduced the use of pesticides dramatically. This decreases the environmental impacts of pesticides and saves farmers many thousands of dollars. Australian regulators have reported no adverse effects over 15 years of Bt cotton use in Australia. Cotton seed oil extracted from Bt cotton can be sold without GM labelling as the extraction processes separate the oil from the plant's proteins and nucleic acids. Therefore, the oil does not have any GM components.

Golden rice

FIGURE 8.1.15 Golden rice is yellow due to the presence of pro-vitamin A.

Vitamin A is a coenzyme that is essential for healthy eyes and the immune system. Diets poor in vitamin A contribute to the deaths of many children in developing countries each year. Vitamin A deficiency can lead to preventable blindness in children and increases the risk of common infections such as measles and diarrhoea.

Rice is a staple food for millions of people around the world. Traditional white rice varieties grown in countries such as Vietnam, Bangladesh and the Philippines are low in vitamin A. Golden rice is a transgenic rice crop that contains pro-vitamin A (Figure 8.1.15). Golden rice is produced when two plant genes and one bacterial gene are inserted into the white rice genome. These genes switch on a biochemical pathway in the rice plant that sends vitamin A to the rice grains rather than the leaves, as would occur in non-modified rice. The transgenic golden rice has been provided to some malnourished communities by aid agencies.

Australian researchers are exploring other genetic modifications to improve the ability of rice plants to use nitrogen for improved growth and reduce the need for nitrogen fertiliser. They also aim to make rice grains richer in elements such as iron, zinc and phosphorus, a process called biofortification.

Issues related to genetically modified organisms

GMOs are controversial for a number of reasons. Debates surrounding their biological, social and ethical implications are common in the scientific and general media. Some of the issues surrounding GMOs are discussed below.

Biological implications

A number of potential biological problems relating to biotechnologies have been raised.

Biological impacts on the health of the organism carrying a foreign gene

Crops approved for commercial growth appear relatively unaffected by a transgene (apart from the new trait). Transgenic animals may experience more adverse effects of transgenes that affect growth rates. For example, growth hormone transgenic pigs are at higher risk of developing arthritis, abnormal skeletal growth and gastric ulcers.

Risks of eating GMOs

One argument against GMOs is the claim that eating these foods will change our DNA in unpredictable ways. There is no reason to think this is the case, as animals have been eating the DNA of plants and other animals for millions of years and this DNA does not result in any change to the core genome of the consumer. There is currently no evidence to support this claim.

Uncontrollable pest plant species

Crops that have been modified for herbicide or insect resistance may breed with other plants, producing a hybridised pest species that farmers may not be able to control. Plants do not usually pollinate different species, so this is only a potential concern for cross pollination with wild relatives, such as between GM canola and wild canola (Figure 8.1.16). Uncontrollable growth would be a concern if the genes code for rapid growth and there was a selective advantage to having the genes in a wild environment.

Cross pollination between GM crops and non-GM crops

Pollen distributed by wind and insects can transfer genes between neighbouring plots of the same species. Cross pollination may happen in both directions and impact both crops. Figure 8.1.17 shows the investigation of contamination by GMO grass in the wild. Genetically engineered creeping bentgrass resists common herbicides for golf courses, allowing weeds on the golf course to be controlled without harming the course grass. The investigation revealed that wind pollination resulted in cross breeding between the genetically engineered grass and wild species. The herbicide-resistant gene was identified in wild grass up to 14.5 km from the origin of the genetically modified grass within the first year of the GM grass being planted.

FIGURE 8.1.16 Cross pollination by insects or the wind could allow this GM canola to grow freely outside its boundaries and become a pest species.

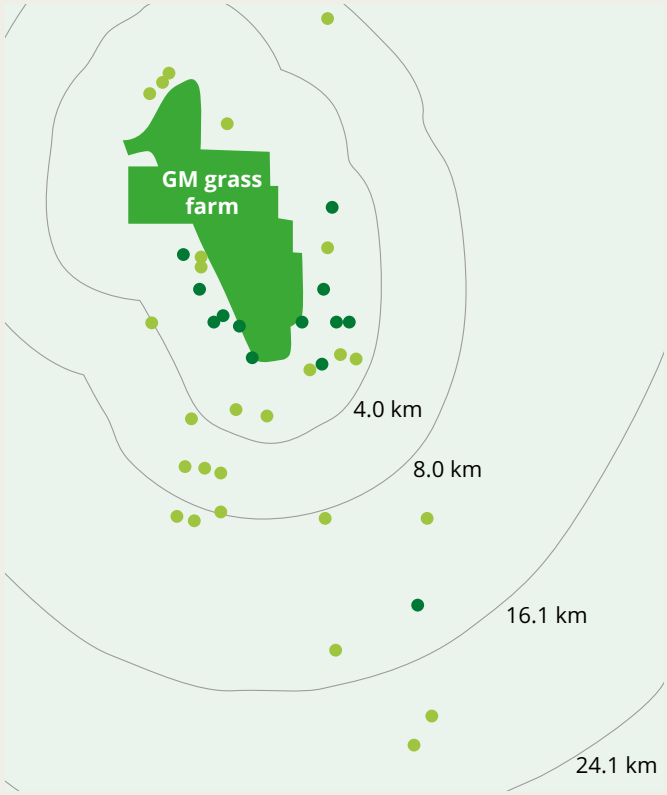

FIGURE 8.1.17 A genetically modified creeping bentgrass farm showing established cross breeding between the GM grass and wild grass (dark green dots) beyond the farm. The light green dots are tested samples that showed no cross breeding.

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Genetically altered animals interbreeding or competing with natural populations

This may be a potential concern if there are wild relatives of the GM animals (animals usually do not breed with unrelated species) and there is a selective advantage to the presence of the transgene in a wild population. For example, GM salmon escaping from aquaculture enclosures adjacent to the ocean could breed with wild salmon. This would introduce a beneficial gene into the wild population, potentially allowing offspring from GM salmon to outcompete the wild salmon, forever altering the genetic make-up of the population. This may then have further implications for the ecosystem (for example, over-predation of small fish and krill that salmon eat).

Loss of biodiversity

As more farmers use GM crops or animals, there will be fewer crops grown that vary from these, reducing the genetic pool of some species. If disease or environmental change occurs, there could be widespread and catastrophic effects on food production. However, this is already a current issue in modern agriculture where monocultures and commercial practices have reduced genetic diversity. GM technology may enhance the trend or it could be used to reintroduce characteristics lost from wild relatives.

Most of these biological concerns apply equally to livestock and crops that have been selected and bred by traditional breeding methods as well as by GM technology.

Social implications

There are a number of social implications relating to biotechnologies.

Solve malnutrition and hunger

GM crops could improve the nutrition and yield of foods already grown by farmers. This could lead to improved health and result in greater availability of food to some of the poorest people in the world who, at the same time, would continue to use their traditional and well-established farming practices and therefore maintain their livelihoods. Although this is good in theory, some people suggest that the best way to end hunger and malnourishment is to find a political solution to inequitable global food distribution rather than rely on agricultural technology such as GMOs.

Create more social inequality

Many of these technologies are expensive but they also have the potential to bring about great benefits. Those that stand to most benefit from these technologies, such as people living in developing countries or experiencing food shortages, may not have sufficient money to pay for patents and associated costs of growing GMOs. A concern is that limiting access to GMOs through patents and pricing bring about greater social inequalities. However, this concern does not appear to have limited the uptake of GM technologies, and many developing countries have embraced GM food and fibre crops while some developed countries oppose their introduction.

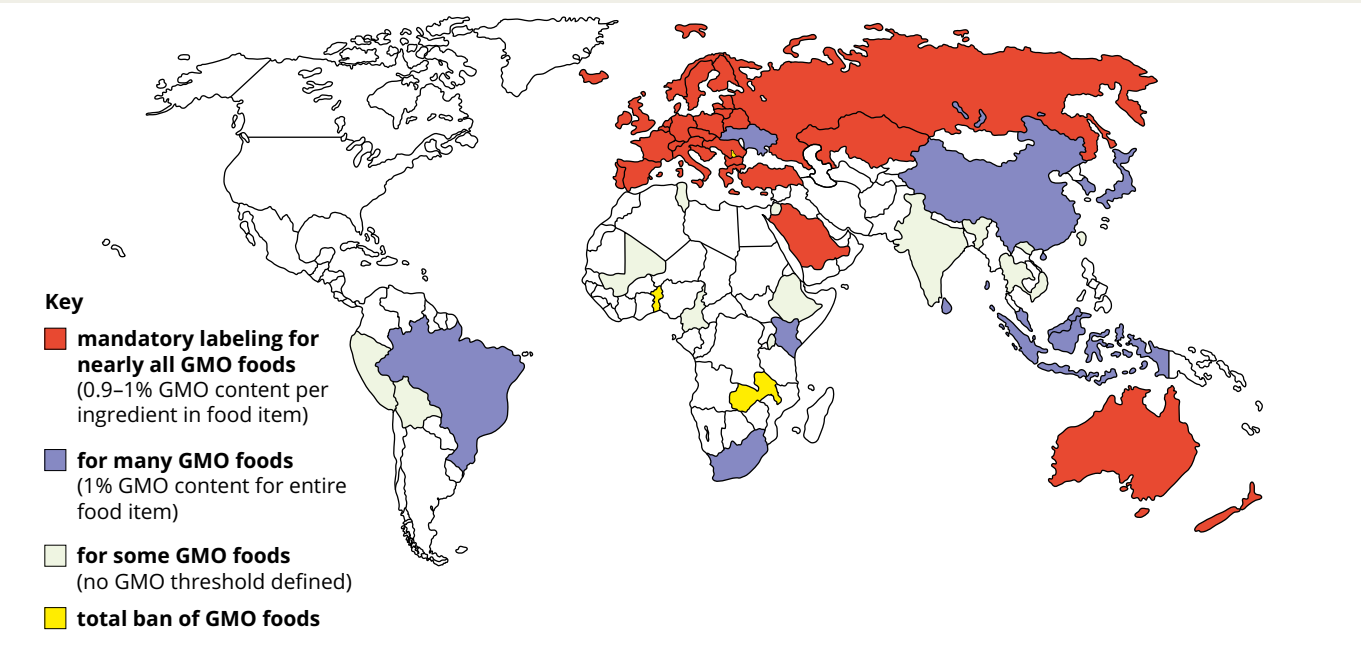

FIGURE 8.1.18 Countries with genetically modified organism (GMO) food labelling laws.

Consumer rights and choice

In more than 60 countries, manufacturers are required by law to disclose on their labels if GMOs have been used in the product. Some countries mandate reporting GMO content at higher levels than others. Australia has some of the world's toughest food labelling laws (Figure 8.1.18), which allows people to make informed choices about what types of food they are buying and eating.

Equal access

It is a human right to have adequate access to food. However, it costs biotechnology companies millions of dollars to research and develop viable, safe GMOs. These biotechnology companies need to see a financial return on their research, yet individuals from developing countries may not be able to afford GM food. Should the biotechnology companies that create the GMOs provide equal access to the technology and at an affordable price for farmers?

Environmental impacts of GMOs versus social consequences

GMOs can have both positive and negative environmental and social consequences, and these must be considered. For example, a reduction in the use of pesticides saves money, which may be invested in education. A decrease in diseases due to GM-insect vector control improves the health of the whole community, enabling greater investment in economic, educational and social development. However, as discussed above, not all farmers can afford to use GMOs, and these farmers may lose their livelihood. Another concern is that GMOs will escape into the wild and introduce modified genes into wild populations, which may have consequences for local ecosystems.

Changes to business practices

Changes in herbicide and insecticide usage affect sales, labour requirements and employment. GM insect-resistant crops no longer require the use of insecticide, saving farmers time and money. However, to grow GM crops, farmers must buy specific seeds and chemicals, which locks them into a specific market.

Ethical implications

GMOs, including transgenic crops, violate the ethical, philosophical and religious principles of some people. Some of these ethical concerns are discussed below.

Patents

If companies control the rights to the genome of GM crops, they also control the prices of the seeds. Farmers have to spend money each year to buy seeds for their crops. For example, the Bt cotton seeds are genetically engineered to be infertile after one generation so farmers may have to purchase new seed each year. This may limit the farmer's ability to make a living.

Cross contamination of a GM crop with a non-GM crop occurs naturally when pollen and seeds are carried by wind or animals. There is the potential for companies owning patents for GMOs to sue farmers whose crops become contaminated by non-GMOs for breaching commercial agreements. The opposite can also happen when organic crops become contaminated with GM crops and the farmer loses the ability to be certified as organic (Figure 8.1.19).

FIGURE 8.1.19 Farmers on this organic canola farm have had their organic crops contaminated by GM canola seeds blown in the wind from the neighbouring property. As a result, they have lost their organic certification and their livelihood. They are in no financial position to challenge the government on decisions made to relax the GM regulations with respect to canola.

Animal rights

Some people believe that producing transgenic animals violates the fundamental rights of an animal. Some believe that using transgenic animals as a source of human proteins or organs is immoral and against animal rights. Animals are already a source of food for humans, so the judgement to be made is whether adding a gene changes these issues.

Some people consider that introducing human genes into animals, for example, to produce pharmaceuticals, makes the animal closer to humans and deserving of the rights and protections given to humans. A question to think about is whether one gene can change the identity of an organism.

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Animals produced through genetic modification may have characteristics that are seen as beneficial to humans and agricultural productivity but may be detrimental to the organism's welfare. For example, some transgenic pigs grow very quickly and this affects their heart and joints, and they cannot be as active as normal pigs.

Intervention in evolution

GM technology expands our ability to interfere with and override normal evolutionary processes. Natural selection processes take hundreds of generations, but GM technology allows humans to immediately introduce altered genes into a population, rapidly speeding up artificial evolutionary processes.

Summary

Figure 8.1.20 summarises the social, biological and ethical implications arising out of the use of GM and transgenic organisms.

Review

1. Discuss the benefits and consequences of using GM crops compared with traditional crops in the fight against vector-borne diseases.
2. After years of farmers spraying pesticides onto crops, the chemicals enter the water cycle and are eventually found in the run-off from farms, including surrounding waterways (e.g. streams, rivers, water supplies and outlets into oceans). Comment on the environmental impact that pesticide-resistance GM crops will have on the local ecosystems of waterways linked to farm run-off.
3. Comment on the impact on plant biodiversity in neighbouring ecosystems of farms using GM crops that are resistant to pesticides and herbicides. Only consider the first few years.
4. Describe one example of a genetic modification that:
   - **a** leads to a reduced environmental impact
   - **b** impacts on both the viability of an animal and the benefit to human health.

social implications

- increased food supply, nutritional content and food quality
- expanded range for growth of agricultural species
- access to the technology; social equality/inequality
- labelling and consumer choice
- patents and pricing; control of access by biotechnology companies
- costs to farmers

biological implications

- safety of consuming GMOs
- cross-pollination between GM plants and wild plants
- cross-pollination between GM and non-GM crops
- viability of transgenic organisms in the wild
- health of GMOs
- genetic variation in agriculture

ethical implications

- violation of animal rights
- human self-interest overrides ethical treatment of other organisms
- intervention in evolutionary process

FIGURE 8.1.20 Summary of some issues arising from the use of biotechnology.

8.1 Review

SUMMARY

- Bioinformatics uses specialised software (such as BLAST) to organise and store the vast amount of data associated with DNA sequencing studies.
- There are a range of ways that bioinformatics can be applied to improve human health and wellbeing, and to improve industry.
- Information from DNA sequencing helps generate maps for the genome of many important organisms, identifying genes, regulatory sites and gene variants.
- Techniques have been developed to create genetically modified organisms (GMOs) where the genes have been edited (gene therapy) or useful genes have been inserted from different species.
- Transgenic organisms are a subset of GMOs that have had a gene from another organism (transgene) inserted into its genome.
- CRISPR is a gene editing technique that uses bacterial DNA sequences to cut DNA at specific locations. CRISPR stands for 'clustered regularly interspaced short palindromic repeat'.

KEY QUESTIONS

Retrieval

1 **a** Define genetic modification and provide an example.

b Describe a successful application of genetic modification in agriculture in recent years.

2 Recall the Australian regulatory body that oversees the development, use and commercial or medical introduction of GMOs.

3 Give an example of how genetic modification may be used to change a characteristic in:

a an animal.

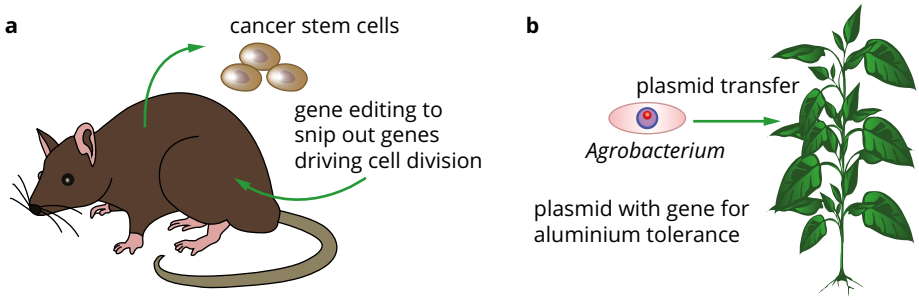
b a crop plant.

List any potential benefits or disadvantages to the organism, the environment or society.

Comprehension

4 Describe a GMO and compare it to a transgenic organism.

5 The following diagram illustrates two model organisms used in research and the molecular procedures being used to alter a genetic characteristic. State whether the resulting organism is genetically modified, transgenic or both.



Analysis

6 Explain why using guide sequences for the CRISPR technique (gRNA) of 20 bases long is problematic. Consider a solution.

8.2 Recombinant DNA, PCR and electrophoresis

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- explain how restriction enzymes function
- describe the process of making recombinant DNA including:
  - the isolation of DNA and cutting of DNA using restriction enzymes
  - the insertion of DNA fragments using plasmid vectors
  - joining DNA with DNA ligase
  - the amplification of recombinant DNA using bacterial transformation
- explain the purpose of the polymerase chain reaction (PCR) and gel electrophoresis
- appraise data from a current genetic biotechnology technique to determine its success rate.

RESTRICTION ENZYMES

DNA molecules are far too long for biologists to work with in their entirety. The discovery and isolation of **restriction enzymes** (also referred to as endonucleases) has enabled scientists to cut DNA into smaller, more usable fragments and isolate single genes. Restriction enzymes are a large group of enzymes that cut DNA at specific recognition nucleotide sequences, known as restriction sites, into smaller fragments. Restriction enzymes occur naturally in bacteria and form part of a bacterial cell's defence system (for example, the CRISPR system). They target foreign DNA that may enter the cell, such as the DNA of **bacteriophages**, cut the foreign DNA into smaller pieces, destroy it and prevent it from replicating.

EcoRI is an example of a restriction enzyme used in molecular biology (Figure 8.2.1). It is isolated from strains of *Escherichia coli* bacteria.

A bacteriophage is a virus that lives within a bacterium, replicating itself and eventually destroying the bacterial cell (sometimes called phage).

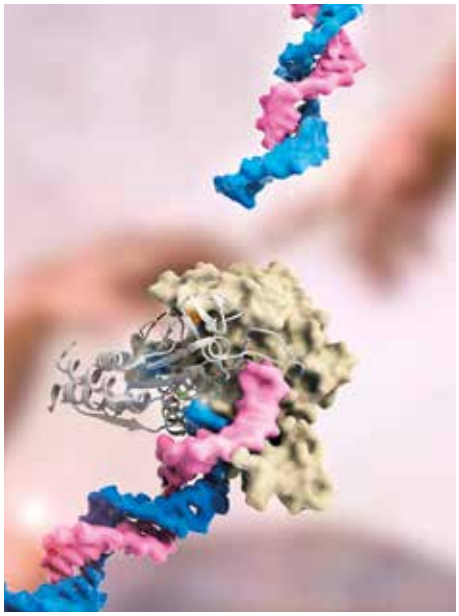


FIGURE 8.2.1 Computer model showing the restriction enzyme EcoRI (grey) cutting a DNA strand (blue and pink).

The first three letters of the name of a restriction enzyme identify the bacterial species from which it was isolated. The fourth letter refers to the particular strain of bacteria. A roman numeral is also included if more than one restriction enzyme has been isolated from this bacterial strain.

The restriction enzyme EcoRI comes from *Escherichia coli* strain RY13 and was the first restriction enzyme isolated from that strain of bacteria.

Each restriction enzyme targets a specific sequence of bases in the polynucleotide chain, usually four to six base pairs in length. The sequence targeted by a restriction enzyme is called a **recognition site**. Every time a restriction enzyme passes its recognition site, it breaks the phosphodiester backbone on each DNA strand, cutting the DNA molecule into fragments of different lengths.

Restriction enzymes of bacterial cells do not cut their own DNA. Bacteria have an enzyme called methylase that adds a methyl group to a specific nucleotide within the recognition site of the restriction enzymes made by the bacterium. This blocks the restriction enzymes from binding to and cutting its own DNA.

Over 800 restriction enzymes have been isolated from bacteria. These enzymes recognise and cut more than 100 recognition sites.

Types of restriction enzymes

There are two types of restriction enzyme. Each cut DNA differently:

- sticky-end restriction enzymes
- blunt-end restriction enzymes.

Sticky-end restriction enzymes

Sticky-end restriction enzymes leave DNA fragments with overhanging ends. They cut the DNA backbone at a different location on each strand within the recognition site. This results in a staggered cut, leaving two fragments with exposed bases or 'sticky ends'. The exposed bases are then able to form complementary base pairs through hydrogen bonding with nucleotides of other DNA molecules that have complementary sticky ends.

EcoRI is an example of a sticky-end restriction enzyme (Figure 8.2.2). It cuts the recognition site GAATTC between the G and A nucleotides on each strand. The sequence on one strand is GAATTC and on the complementary strand it is CTTAAG (the same sequence read backwards). This is called a palindromic sequence. EcoRI cuts at a different location on each strand, so sticky-end fragments are produced.

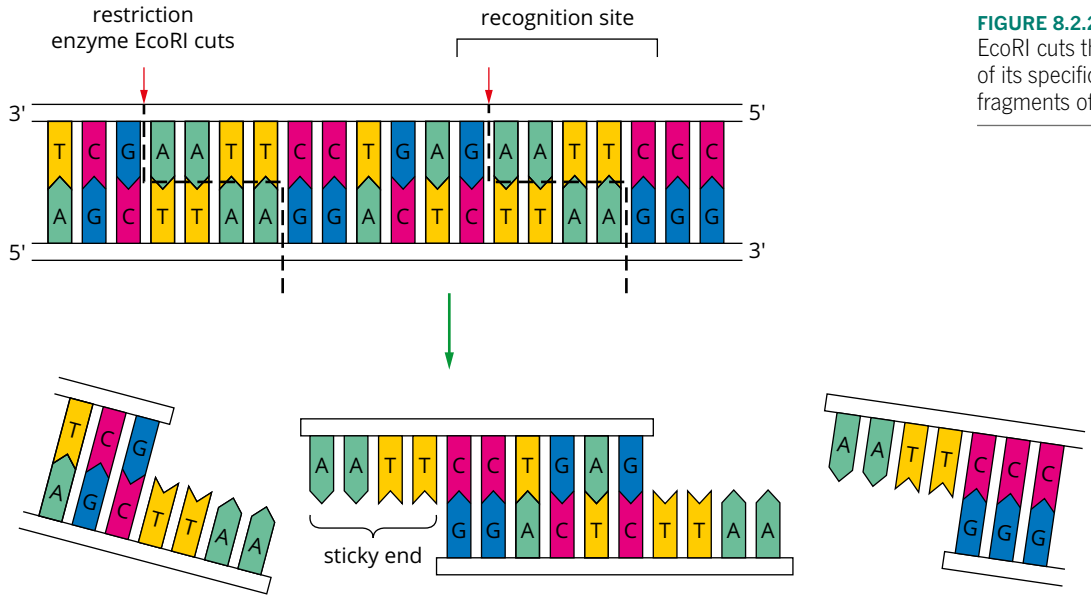


FIGURE 8.2.2 The sticky-end restriction enzyme EcoRI cuts the DNA between the G and the A of its specific recognition site, GAATTC, creating fragments of DNA with sticky ends.

Blunt-end restriction enzymes

Blunt-end restriction enzymes leave clean-cut ends by cutting the sugar–phosphate backbone on both strands of the DNA molecule at the same location within the recognition site (Figure 8.2.3).

HaeIII is an example of a blunt-end restriction enzyme. It is extracted from the bacterium *Haemophilus aegyptius*. It cuts the recognition site GGCC between the G and C nucleotides. These two bases are in the exact same location on either strand of the DNA molecule, resulting in a straight cut through both stands that leaves two fragments with blunt ends.

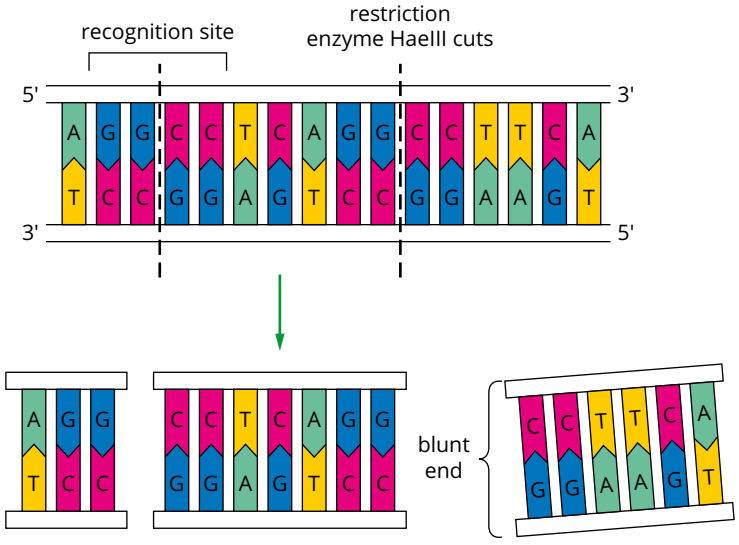


FIGURE 8.2.3 The blunt-end restriction enzyme HaeIII cuts the DNA between the G and the C of its specific recognition site, GGCC, creating fragments of DNA with blunt ends.

Restriction enzymes as tools to identify polymorphisms and mutations

Alleles are variations of genes that exist within a population (Chapter 5). When an allele has at least a 1% frequency in a population it is called a **polymorphism**.

Single nucleotide polymorphisms (SNP) are exactly what their name suggests: a polymorphism (numerous changes) at a single nucleotide. This is in reference to studying the DNA sequence in a large sample of people or population, where the nucleotide at a specified position in the DNA sequence is different throughout the population. Some SNPs are found in non-coding portions of the DNA. They do not change the protein's structure but they can have influences on its regulation or expression. Some SNPs are related to how people respond to particular medications or are linked to an increased risk for specific diseases. Others are causative SNPs, which are directly related to protein function.

In general, restriction enzymes are used to cleave (cut) DNA into smaller fragments for DNA sequence analysis. If an SNP or mutation exists at the cleavage site of the restriction enzyme, the restriction enzyme will cleave the DNA at a different location. This allows the sequence variant can be identified (Figure 8.2.4). By utilising people with the same disease, researchers have been able to find specific SNPs that are related to certain diseases.

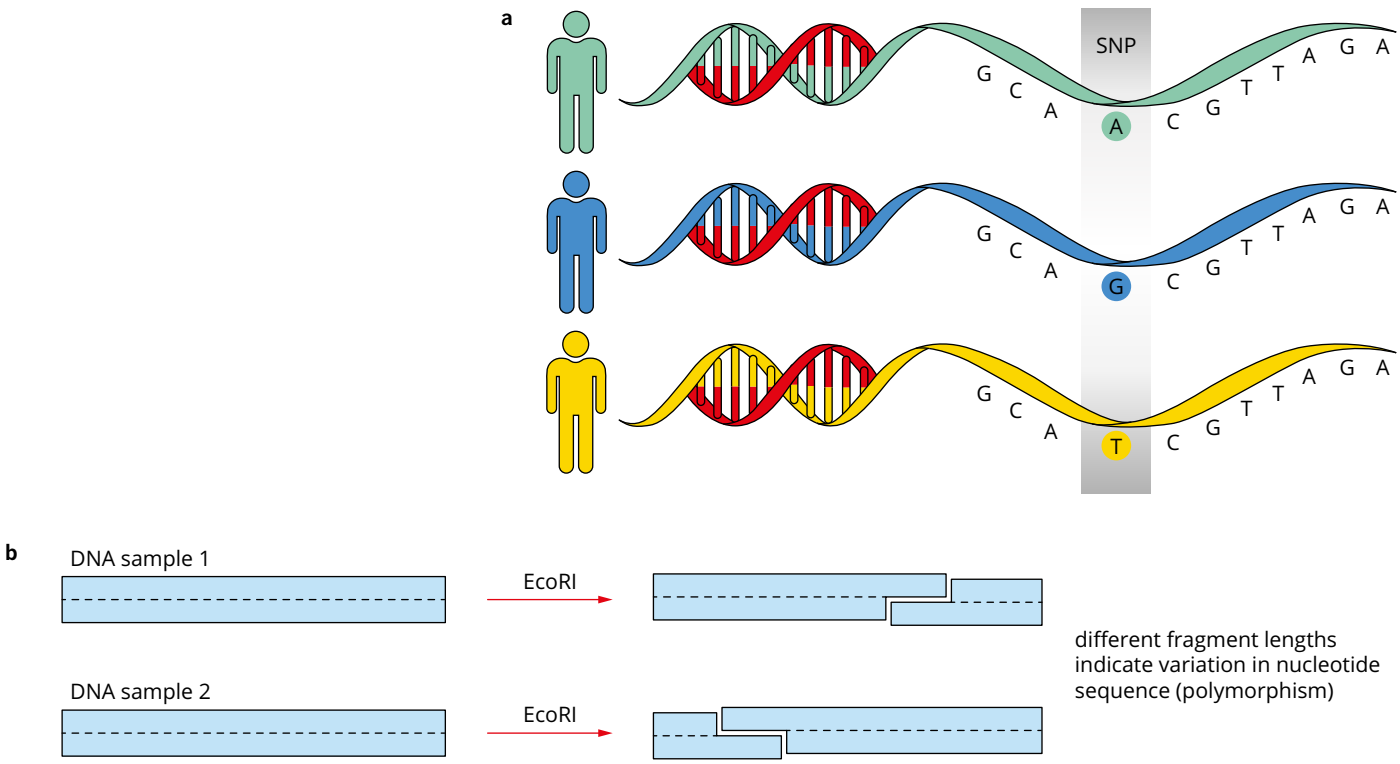


FIGURE 8.2.4 (a) A single change in one base will result in gene variation or single nucleotide polymorphism (SNP). (b) Fragment length will differ with the digestion by a restriction enzyme like EcoRI and the polymorphisms will be easily identified using electrophoresis.

It is hoped that, in the future, diagnosis and perhaps prognosis can be determined using genetic analysis rather than waiting for patients to show signs and symptoms. Table 8.2.1 outlines the estimated risk of type 2 diabetes associated with identified SNPs. It details the genes in which SNPs have been studied, the chromosome they are found on, the nucleotide position within the gene sequence, the normal (non-disease associated) homologous genotype and the SNPs identified. For example, for the *LOC105375716* gene, individuals with the C:C SNP are three times more likely to develop type 2 diabetes than individuals with the normal genotype.

TABLE 8.2.1 SNPs associated with an increase risk in type 2 diabetes

Gene	Chromosome number	Nucleotide position	Normal genotype	SNP
IGF2BP2	3	185 793 899	G:G	G:T (1.2× increased risk) T:T (1.2× increased risk)
CDKAL1	6	20 661 019	G:G	C:G (1.3× increased risk) C:C (1.3× increased risk)
vicinity of *CDKN2B*	9	22 134 095	C:C	C:T (1.2× increased risk) T:T (1.2× increased risk)
LOC105375716	8	117 172 544	T:T	C:T (2.5× increased risk) C:C (3× increased risk)

Ligases

Ligases are a group of enzymes that join fragments of DNA or RNA in a process called **ligation**. **DNA ligase** is used to join fragments of DNA and **RNA ligase** is used to join fragments of RNA. The role of DNA ligase in a cell is to join segments of newly replicated DNA (for example, Okazaki fragments) and repair breaks in DNA molecules.

Assuming they were cut with the same restriction enzymes, DNA ligase can join DNA fragments extracted from different organisms or different species, due to DNA's universally consistent molecular structure. Depending on the characteristics of the DNA ends to be joined (sticky or blunt ends), the conditions of the ligation reaction (incubation time and temperature) need to be adjusted to ensure efficient DNA ligation is achieved.

Ligation of sticky-end fragments

Ligation to join sticky-end fragments is specific because the exposed bases of sticky-end fragments first bind by complementary base pairing. Complementary bases are attracted by weak hydrogen bonds that hold them together. After this, the ligase joins the fragments (Figure 8.2.5) by creating a phosphodiester bond between the 3' and 5' ends of the adjoining nucleotides. This technique makes recombinant DNA and is used in processes such as **gene cloning**.

Ligases join two DNA fragments together by creating a phosphodiester bond between them.

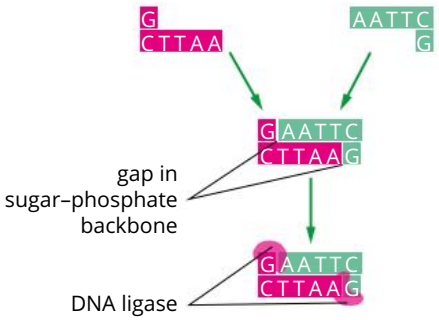


FIGURE 8.2.5 Two sticky-end DNA fragments come together by complementary base pairing. DNA ligase permanently links the sugar–phosphate backbone.

Ligation of blunt-end fragments

Ligation of blunt-end fragments is random. Any two fragments can join if they come in contact and the DNA ligase joins them. For this reason, blunt-end fragments are more difficult to use in DNA manipulation processes that require the joining of specific fragments (Figure 8.2.6). However, blunt ends are sometimes unavoidable. For instance, a blunt-end enzyme might be the only type available to cut out the target gene without damaging the gene itself. Using DNA ligase, scientists are able to attach short DNA fragments onto blunt-end DNA to create sticky ends.

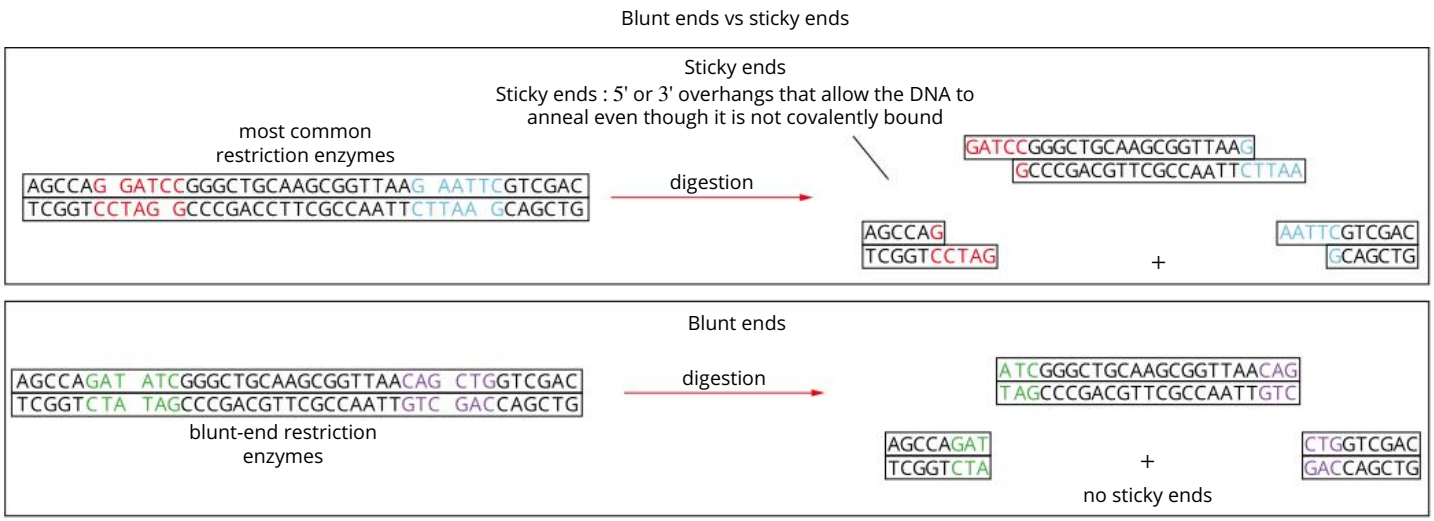


FIGURE 8.2.6 Blunt-end joins are more difficult to 'stick', especially for larger segments of DNA.

RECOMBINANT DNA

When DNA from two different sources is joined together, the resulting molecule is called **recombinant DNA**. Scientists create recombinant DNA to clone (make multiple copies of) a particular gene. Following this step, they may also produce large quantities of the protein expressed by the cloned gene. For example, an insulin-coding gene may be cloned and then incorporated into bacteria to produce large quantities of the protein insulin to be used as treatment for diabetes. The creation of recombinant DNA often requires the use of plasmids.

A range of proteins for therapeutic and industrial purposes are produced using recombinant DNA technology. Besides insulin, other therapeutic examples include epidermal growth factor used in the treatment of burns to improve the survival of skin grafts, interleukin-2 used in cancer treatment, antibodies for immunotherapy and vaccines against a number of viruses. Industrial examples include enzymes such as amylase, lipase, protease and cellulase used in food processing, the textile industry and as detergent additives.

Plasmids

In eukaryotic cells, DNA forms long, linear chromosomal strands containing thousands of genes. In prokaryotic cells, DNA forms double-stranded circular chromosomes.

In addition to the circular chromosome, bacterial cells also contain small circular pieces of double-stranded DNA called plasmids. Plasmids replicate independently of the chromosome. As bacterial cells do not have a nucleus, both chromosomal and plasmid DNA are located in the cytosol (Figure 8.2.7).

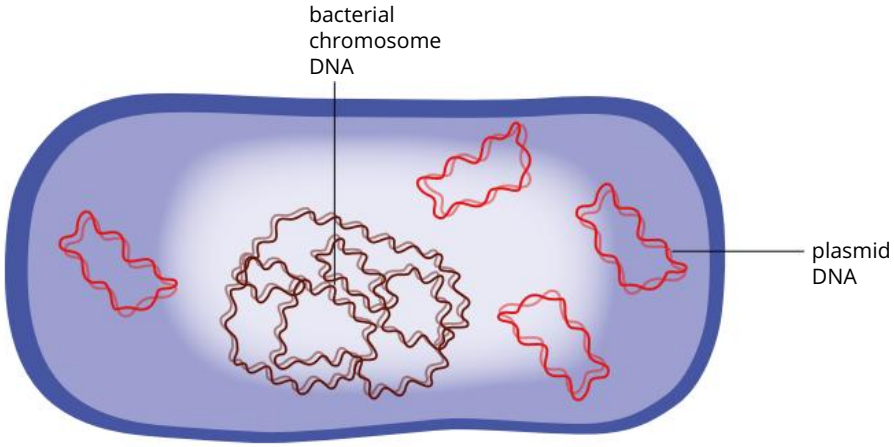


FIGURE 8.2.7 Bacterial cells contain a circular bacterial chromosomal DNA (brown) and small circular pieces of DNA called plasmids (red).

Using plasmids as vectors

When scientists create recombinant DNA, they often use a plasmid as the vector. They insert target DNA into the plasmid, producing a **recombinant plasmid**. The plasmid is then placed in a bacterial cell, where the self-replicating system of the plasmid and cell replicates the plasmid genes. Each bacterial cell containing the plasmid will produce the protein products of the genes in the plasmid (including those of the target DNA). For example, if a recombinant plasmid containing the insulin-coding gene is placed in a bacterial cell, the cell will produce insulin.

Plasmids are used as vectors when creating recombinant DNA for the following reasons:

- Their small size makes them easy to manipulate in a laboratory.
- Plasmids carry a range of restriction enzyme sites. A plasmid containing the appropriate recognition sites is chosen to suit your needs. For example, if your gene of interest was cut from a chromosome with EcoRI, you would use a plasmid with a single EcoRI site.
- Recombinant plasmids self-replicate independently once they are placed inside a host bacterial cell and at a faster rate than their bacterial host's chromosomal DNA. This is very important when manufacturing vast quantities of proteins.

To enable the identification of cells that have incorporated the recombinant plasmid, the plasmids used as vectors need to have particular characteristics, such as:

- an antibiotic resistance gene
- a gene that can be easily identified, such as a gene that produces coloured or fluorescent proteins.

Green fluorescent protein

Green fluorescent protein (GFP) is a protein that was first isolated from sea jellies. The *GFP* gene can be inserted into plasmids to be used in bacterial transformation. Bacterial cells that have incorporated this plasmid vector fluoresce green under UV light.

Genes that code for fluorescent proteins are often attached to another gene that is being investigated. When this gene of interest is expressed, the fluorescent protein is also produced, so it sends an obvious 'visual report' that the gene is being expressed (Figure 8.2.8).

FIGURE 8.2.8 Transformed bacterial colonies can be identified as they contain the *GFP* gene and fluoresce under UV light.

Creating recombinant DNA

The process of creating a recombinant plasmid is outlined in the four steps below and in Figure 8.2.9:

1 The target DNA is cut out using a sticky-end restriction enzyme and then isolated.

2 The bacterial plasmid is cut by the same restriction enzyme used to cut out the target DNA. The plasmid and the target DNA now have the same sticky ends with exposed bases that are complementary to each other.

3 The target DNA and plasmids are placed together. Some plasmids will simply close up again (known as non-recombinant plasmids), while other plasmids will incorporate the target DNA by complementary base pairing (known as recombinant plasmids).

4 DNA ligase is added to rejoin the sugar–phosphate backbone of the DNA.

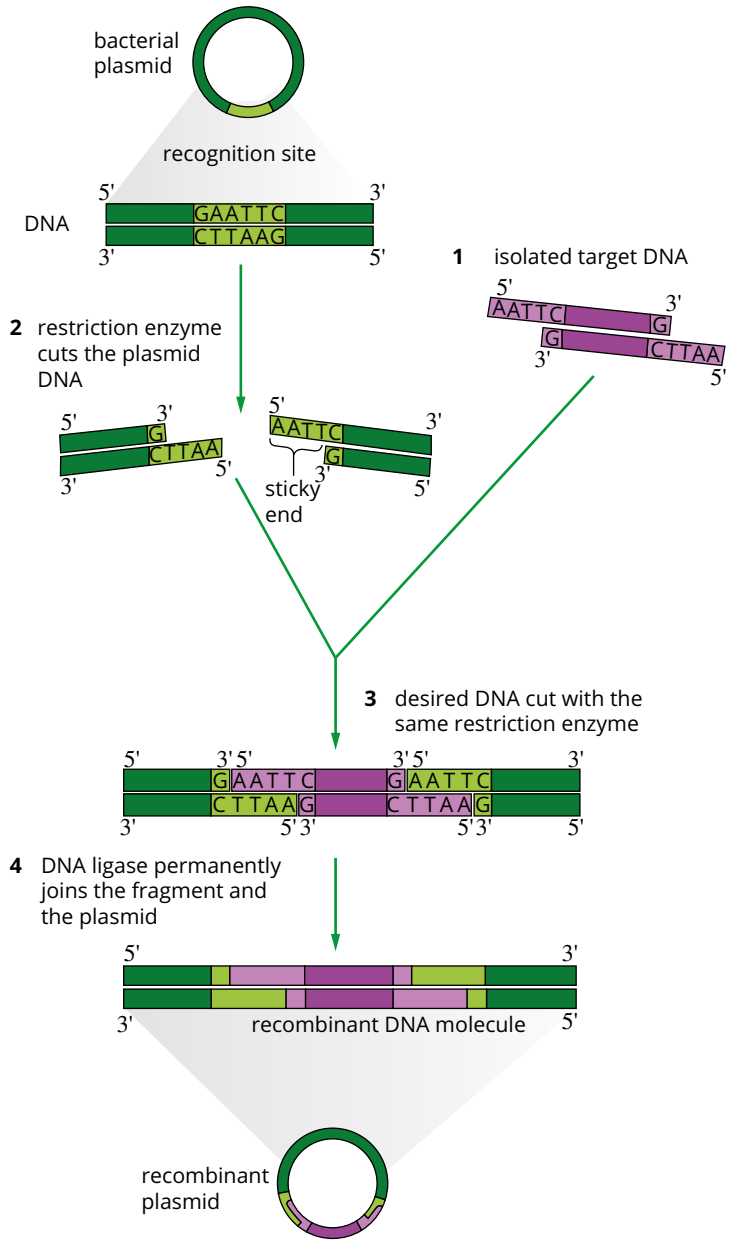


FIGURE 8.2.9 A recombinant plasmid is created by joining a target DNA fragment and a plasmid that have both been cut with the same sticky-end restriction enzyme. They are joined using DNA ligase.

GENETICALLY TRANSFORMING CELLS

The DNA in cells can be vulnerable to attack from the environment, whether it is safe hiding in the nucleus of a eukaryotic cell or scattered about the cytoplasm in a prokaryotic cell. Viruses infect nearly every cell type and incorporate their DNA or RNA into the host's genome. **Transposons** (or jumping genes) were identified in maize in the 1940s. These were the first mobile eukaryotic genes investigated. The existence of transposons suggests that DNA is a mobile and complex element, which challenges the notion that DNA remains in the nucleus, rigidly controlled by the translation process.

Cells that have had foreign DNA incorporated into them are said to be **transformed**. For example, when a foreign plasmid is incorporated into a cell, the cell is transformed because it can express a new gene and therefore has a new characteristic. Genetic transformation can be both natural and artificial.

Transforming prokaryotic cells

Prokaryotic cells can be transformed by exchanging genetic material naturally, or through artificial means such as adding foreign DNA or RNA to host cells (**transfection**).

Natural transformation

Bacterial cells exchange genetic material naturally through several methods. This is how an organism that reproduces asexually by splitting in two can evolve and spread antibiotic resistance.

Bacterial **competence** refers to the ability of a bacterial cell to alter its genetic make-up by taking in extracellular DNA. A double strand of DNA is usually too large to move across the cell membrane, so the double strand is broken down to two single strands. One of the single strands is brought into the cell, while the other one is degraded by bacterial restriction enzymes. The strand that is taken in by the bacterial cell is integrated into its genetic material and copied accordingly. For example, certain strains of *Streptococcus pneumoniae* seek out other sister cells and unrelated species of bacteria found in the upper respiratory **biofilm**, **lyse** (or burst) the cells, then take up their DNA.

Bacterial competence contributes to the development of antibiotic resistance, as bacterial cells can take in antibiotic resistance genes from other bacterial cells. Since antibiotic resistance in potentially deadly superbugs is of urgent interest to scientists, bacterial competence is being researched more thoroughly.

Artificial transformation

Two methods of artificial **bacterial transformation** are used in the laboratory: heat shock and electroporation.

Heat shock involves placing bacterial cells and a mixture of recombinant and non-recombinant plasmids in an ice-cold solution containing calcium ions and then rapidly increasing the temperature to disrupt the plasma membrane of the bacterial cells. The plasmids can then penetrate the membrane and enter the bacteria.

In **electroporation**, the bacterial cells and a mixture of recombinant and non-recombinant plasmids are subjected to an electrical current that alters the plasma membrane. Again, the plasmids are then able to enter the bacteria.

Very few of the bacterial cells will be transformed with recombinant plasmids using either of these two methods. Some will take up the non-recombinant plasmids (plasmids without the target DNA) and others will not be transformed at all or will die from the heat shock or electroporation treatment (Figure 8.2.10 on page 402). As long as a small number of cells transform, a population or culture can be grown from these.

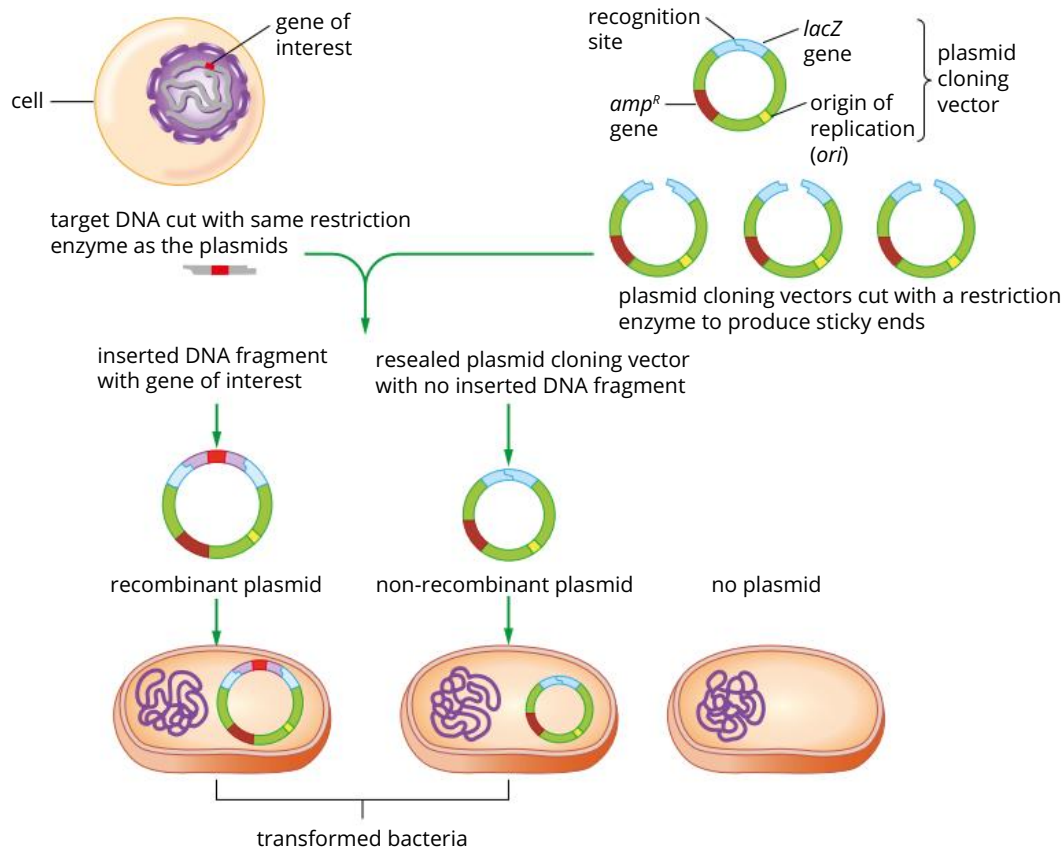


FIGURE 8.2.10 The process of bacterial transformation involves creating recombinant plasmids and then inserting them into bacterial cells. Some will successfully take up the recombinant plasmid but others will either contain a non-recombinant plasmid or no plasmid at all.

Transforming eukaryotic cells

Bacterial transformation is used in crop biotechnology to transfer genes into plants and introduce a desirable trait, such as increased productivity, salt tolerance, insect resistance or herbicide resistance. Such plants are called transgenic (Module 8.1). The bacterium *Agrobacterium tumefaciens* is commonly used as it has the ability to both infect plant tissue and incorporate specific parts of its plasmid into the host plant's DNA.

Natural transformation

The study and use of *A. tumefaciens* has a long history. This common soil bacterium was identified as the cause of the tumours of crown gall disease more than 100 years ago. A plasmid that can move from the bacterium into plant cells was later identified as the key agent. This is a natural transformation of plant cells by a bacterial plasmid. The plasmid in *A. tumefaciens* is called the Ti or tumour-inducing plasmid (Figure 8.2.11). It has genes that direct the movement of the plasmid from the bacterium into plant cells and for insertion into the chromosomes of the plant. It also has genes for growth-promoting plant hormones that cause tumours.

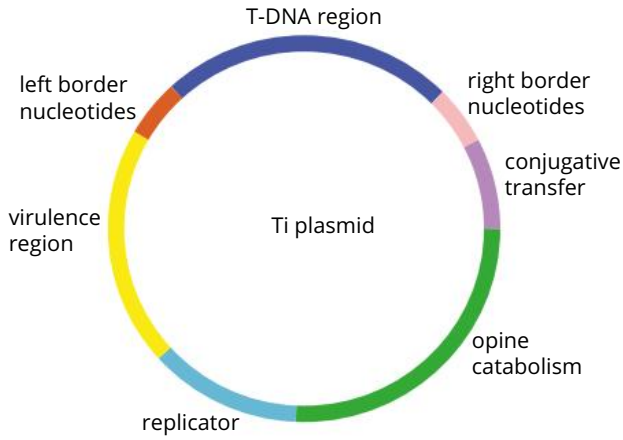


FIGURE 8.2.11 The Ti plasmid from *Agrobacterium tumefaciens*. The T-DNA region carries the genes that cause tumours in crown gall disease. This region is removed and replaced with a foreign gene, making a recombinant plasmid.

This plasmid is used for genetic engineering. The genes that cause tumour growth are removed while the genes that ensure transfer from bacterium to plant cell remain. A foreign gene inserted into this plasmid can then be transferred into plant cells. Genes for traits such as insect resistance, herbicide resistance and tolerance to salinity and frost are all being used in crop biotechnology.

The foreign gene is inserted into the plasmid, then *A. tumefaciens* is transformed by the methods described earlier. Transformed bacteria and plant material are mixed (Figure 8.2.12a). *A. tumefaciens* inserts a section of its plasmid (containing the desired gene) into the plant cell, which is then incorporated into the plant cell DNA. This plant tissue is cultured (Figure 8.2.12b) to create a transgenic plant that expresses the foreign gene.

FIGURE 8.2.12 (a) Plant material is exposed to *Agrobacterium* to allow transfer of recombinant plasmid from bacteria to plant cells. (b) The plants carrying the new gene are cultured and selected in the laboratory before release for field testing of the new characteristics acquired by gene transfer.

In Queensland, transgenic cotton is created using bacterial transformation to give insect and herbicide resistance. The crops can be sprayed with a weed killer that does not kill the cotton but removes the competing weeds from the paddocks.

Artificial transformation

Most animal cells undergo transfection. Some methods include viral vectors, the use of a gene gun to shoot beads coated with DNA into plant cells and **microinjection** of the gene into the **pronucleus** of a reproductive cell (Figure 8.2.13). Electroporation can also be used (as outlined with prokaryotes earlier).

The use of eukaryotic cells as GMOs (Module 8.1) is very important, as bacteria are limited in their ability to produce functioning eukaryotic proteins. Many biologically active proteins are glycoproteins, which have sugars attached to the protein chain. Sugars are added in the endoplasmic reticulum and Golgi apparatus of eukaryotic cells. Because bacteria lack these organelles and the necessary enzymes, they are unable to produce glycoproteins. Therefore some recombinant proteins are mass-produced in eukaryotic cell cultures, such as yeast or mammalian fibroblasts. Examples are Gardasil, the vaccine against human papilloma virus, made from recombinant DNA in yeast, and erythropoietin (which controls red blood cell production), produced from recombinant DNA in cultured mammalian cells.

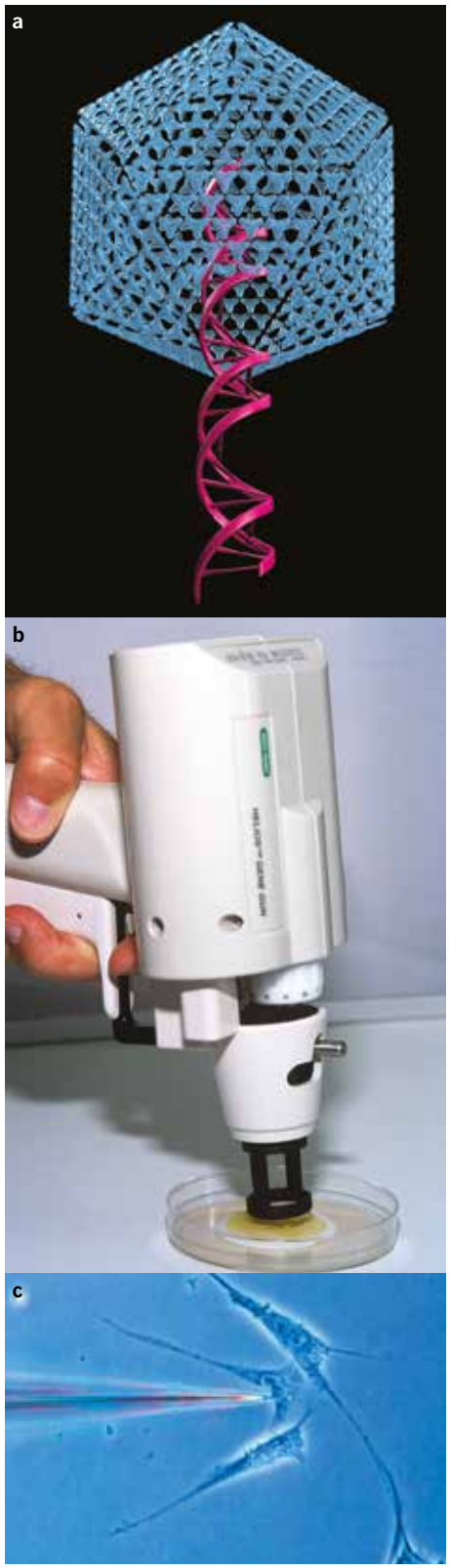


FIGURE 8.2.13 (a) Viral vectors are used to transfer genes into plant and animal cells. (b) The gene gun, a biolistic device, shoots DNA into plant cells. (c) Microinjection of DNA into mammalian cells in a culture dish.

Selection and screening of transformed bacteria

FIGURE 8.2.14 Growth of transformed bacteria on agar plates containing ampicillin and X-gal. Bacteria with the non-recombinant plasmid appear blue because the *lacZ* gene is expressed. Bacteria with the recombinant plasmid appear white.

When determining which bacterial cells have been transformed with recombinant plasmids containing target DNA, the characteristics of the plasmid vectors described earlier becomes important. The earlier example (Figure 8.2.7 on page 399) shows that the plasmid vector contains other genes, including a gene for antibiotic resistance or a gene that displays a particular phenotype, such as a coloured product.

To determine which of the bacterial cells have been transformed, the cells are incubated at 37°C so that they can reproduce and form colonies on agar plates that contain the antibiotic (ampicillin). The only bacteria to survive will be those that have taken up the plasmid, whether it is a recombinant or non-recombinant plasmid. These bacteria have the ampicillin-resistance gene. All other bacteria will be killed.

In this example, the plasmid carries the ***lacZ* gene**, which codes for an enzyme that breaks down an indicator called X-gal, resulting in a blue product. Bacteria carrying the non-recombinant plasmid with an intact and functioning *lacZ* gene produce blue colonies on agar plates. If the target DNA has been successfully inserted into the *lacZ* gene, expression is disrupted and the enzyme coded by this gene is not produced. Therefore, bacteria transformed with recombinant plasmids appear as white colonies (Figure 8.2.14 and Figure 8.2.15).

Bacteria transformed with the recombinant plasmids are then taken from the agar plate and cultured with nutrients in order for them to replicate and produce the protein encoded by the target DNA.

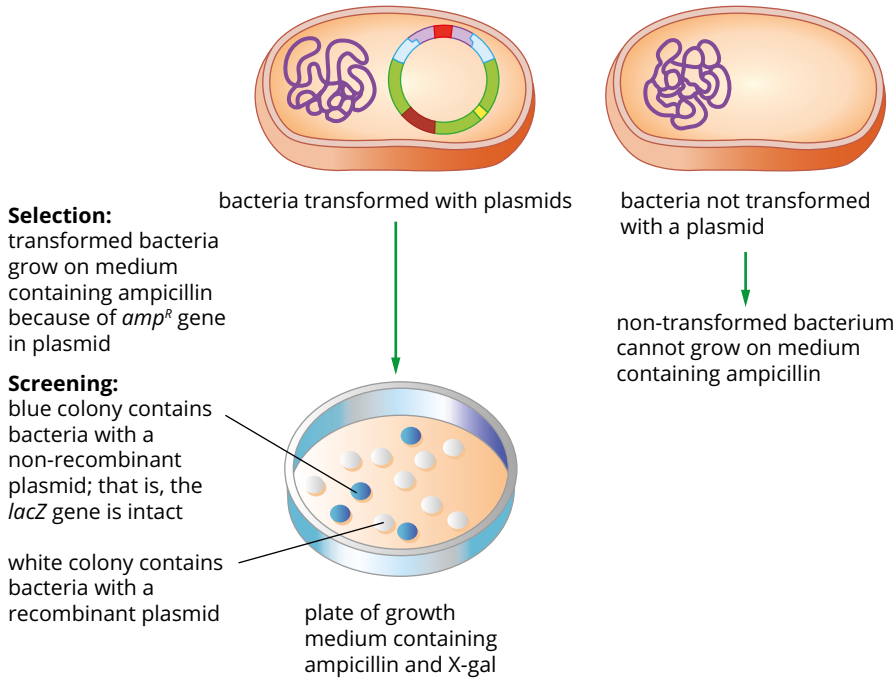


FIGURE 8.2.15 Selection and screening of bacterial cells to identify which cells have been transformed (contain a plasmid), and then which colonies contain recombinant plasmids with the target DNA and which contain non-recombinant plasmids.

WS 4.1.11

DNA AMPLIFICATION

Many DNA manipulation techniques and biotechnology require a large quantity of DNA to work with. However, sometimes only a very small sample of DNA is available to scientists. For example, only trace samples of DNA may be left at a crime scene or extracted from fossils of extinct species (Figure 8.2.16), and only small samples can be removed for medical tests or for use in embryonic or fetal DNA screening for genetic disorders. In these cases, **DNA amplification** is required to increase the amount of DNA so that it is large enough to be used or analysed.

DNA amplification uses the **polymerase chain reaction (PCR)** to create a large quantity of DNA that is identical to the initial trace sample. DNA amplification enables the rapid and accurate replication of target DNA, resulting in millions of copies being produced in a matter of hours. The term 'target DNA' is used to describe a particular region of a DNA molecule that a scientist intends to study or manipulate (for example, a specific gene or a **microsatellite**, which is a variable region of the genome used for DNA profiling).

FIGURE 8.2.16 A scientist extracts fossilised DNA from a Neanderthal bone. The DNA will be amplified for further DNA analysis.

Polymerases

As the name indicates, the polymerase chain reaction (PCR) is based on the action of polymerases. Recall from Chapters 5 and 6 that **polymerases** are enzymes that catalyse the formation of polymers, in particular nucleic acids. There are two different groups of polymerases used in DNA amplification technology:

- DNA polymerase
- RNA polymerase.

Scientists mimic the process of DNA replication in a test tube for techniques such as PCR and DNA sequencing. In PCR, the strands of the double-stranded DNA are separated by heating rather than with a helicase enzyme. To be amplified, each double-stranded DNA sequence needs two **primers**. The primers are made in a laboratory and have a nucleotide sequence that is complementary to each end of the DNA sequence to be amplified. This enables them to target particular regions of a nucleotide sequence within a gene, chromosome or genome. The DNA polymerase enzyme used in PCR has particular properties.

Taq polymerase

Taq polymerase is the DNA polymerase that is most commonly used in PCR. It was originally extracted from the **thermophilic** bacterium *Thermus aquaticus*. The heat-resistant properties of Taq polymerase make it extremely useful in DNA manipulation techniques such as PCR.

Reverse transcriptase

Reverse transcriptase is a DNA polymerase that synthesises single-stranded DNA using single-stranded RNA as a template. This is the reverse of the usual transcription process in which DNA is transcribed into RNA. Reverse transcriptase is used in the laboratory to produce DNA molecules that can be amplified by PCR for further analysis. Reverse transcriptase is also used to make **complementary DNA (cDNA)** from mRNA that has already had the introns spliced out. The cDNA is then inserted into bacterial plasmids so that the protein coded for by the mRNA can be made in the laboratory.

TABLE 8.2.2 Exponential growth in number of target DNA molecules during PCR

Number of PCR cycles (n)	Number of double-stranded copies of original DNA (2^n)
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	125
8	256
9	512
10	1024
20	1 048 576
30	1 073 741 824

Polymerase chain reaction

PCR is carried out in cycles. Each cycle doubles the amount of DNA, resulting in exponential growth. In other words, it is a chain reaction that creates billions of copies of target DNA in a few hours. Table 8.2.2 shows how each PCR cycle increases the amount of target DNA exponentially. After 30 cycles, there are more than 1 billion copies.

For this reaction to be carried out, a PCR mixture is required, containing:

- DNA, including the target DNA to be amplified (the sample) (Figure 8.2.17)
- free nucleotides to build new DNA strands
- a heat-resistant DNA polymerase (usually Taq polymerase) to elongate the new DNA strands by adding the free nucleotides
- two DNA primers complementary to the ends of the target DNA, to specify the start and finish of the DNA fragment to be amplified. The two primers are synthetic, single-stranded DNA molecules up to 30 bases in length. They are specifically created to bind through hydrogen bonding with complementary base pairs on either end of the DNA to be amplified.

FIGURE 8.2.17 A scientist loads DNA samples into a thermocycler to be amplified by PCR.

Steps in the polymerase chain reaction

The PCR mixture is placed in a **DNA thermocycler**, which alters the temperature in preprogrammed steps. Each PCR cycle involves three steps (Figure 8.2.18):

1. Denaturation: The sample is heated to 95°C to break the hydrogen bonds between the two strands of double-stranded DNA to obtain the single strands of DNA.
2. Annealing: The temperature is reduced to 50–60°C. This allows the primers to **anneal** (or bind) to complementary sequences on opposite strands at each end of the target DNA sequence.
3. Extension: The temperature is increased to 72°C. This allows Taq polymerase to attach to the primers on the DNA strands. The Taq polymerase moves along each strand, adding free nucleotides to form double-stranded DNA.

This three-step cycle of heating and cooling is repeated up to 50 times to ensure there is sufficient target DNA produced to work with.

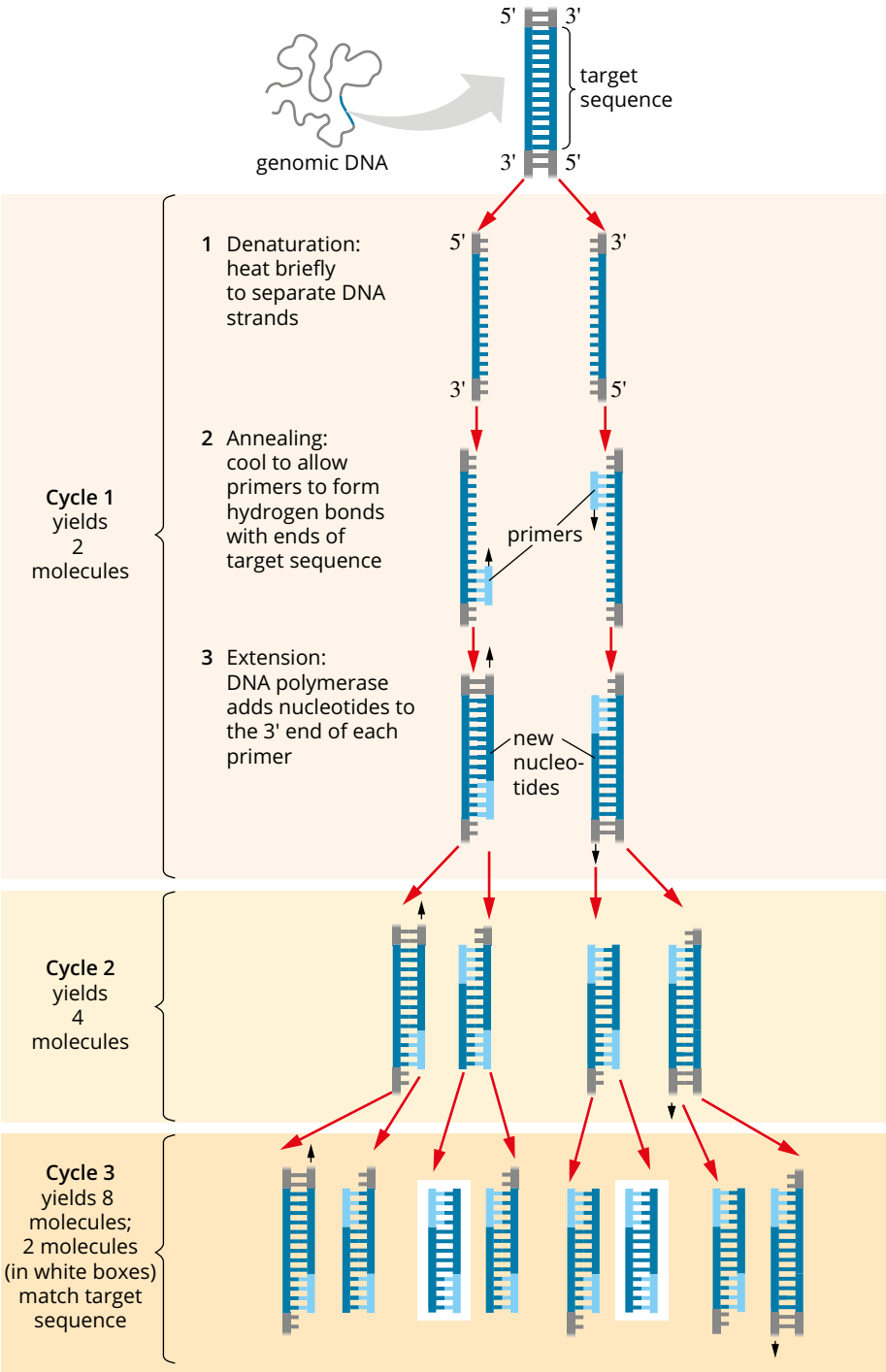


FIGURE 8.2.18 The three steps of PCR: denaturation, annealing and extension. Each PCR cycle increases the amount of target DNA exponentially.

GEL ELECTROPHORESIS

Gel electrophoresis is commonly performed after DNA amplification. Gel electrophoresis allows scientists to separate out the different DNA fragments (formed using restriction enzymes) or proteins present in a sample based on their size. This information can be used to match fragments from other samples, as in DNA profiling, or to isolate a particular fragment for further use in another technique, such as DNA recombination and bacterial transformation.

Samples of a known size are run in an adjacent track so that the unknown samples can be identified against a standard run. When an electric current is applied to the gel, the negatively charged DNA molecules in the gel move towards the positive terminal. Small DNA molecules move faster than large ones, causing them to separate based on their size. A series of tracks depositing the various sized pieces of DNA can be visualised and compared to the standard run in the gel.

Gel electrophoresis is used to compare DNA fragments for a number of applications.

- DNA screening: This compares a number of samples against a control. The control is used as the standard run and a number of samples (known or unknown) are run against the control to see if there are any similarities. Examples of DNA screening include paternity tests, crime scene analysis and comparative analysis between species.
- Confirming the correct gene has been amplified in PCR: Gene amplification is used in many technologies and gel electrophoresis is used as quality control. An example is when a research laboratory purchases a quantity of DNA or genes for research. The provider needs to ensure they have amplified the gene that has been purchased, so they use gel electrophoresis as a quality control measure. The standard run is the known gene and random samples from the amplified product are tested.
- Identifying DNA fragments obtained by restriction enzyme digestion: When a new gene has been isolated, gel electrophoresis provides the banding that characterises the gene. This will then be used as the template or calibration for that gene.

The following steps and Figure 8.2.19 outline the process of gel electrophoresis.

1. An electrophoresis gel is prepared. It has a jelly-like texture and is usually composed of agarose (a purified form of agar). The porous matrix of the agarose allows molecules to travel through. The gel is rectangular and contains small wells (holes) at one end.
2. The gel is placed into a gel electrophoresis chamber with the wells situated at the negative terminal of the chamber.
3. Each DNA sample has been treated with a restriction enzyme to break it into fragments. The samples are loaded into the wells within the gel.
4. A DNA ladder containing DNA fragments of known length is also run on the gel for comparison with the samples. This allows the length of the sample DNA fragments to be estimated.
5. The gel is placed in an electrophoresis bath where it is covered with a controlled pH solution that contains ions to conduct an electric current.
6. A power source is attached to the electrophoresis bath and switched on. The electrical current causes the negatively charged DNA fragments to migrate through the gel towards the positive terminal of the chamber.
7. Smaller fragments move faster through the gel, so they migrate further through the gel than larger fragments in a given period of time. This sorts the fragments by length.
8. The DNA fragments are detected by applying a stain that binds to DNA. This can be done with a fluorescent stain (which may be included in the gel or added after) or with methylene blue stain (which is added with the sample or after running the gel). Fluorescent stains are viewed with ultraviolet (UV) light.

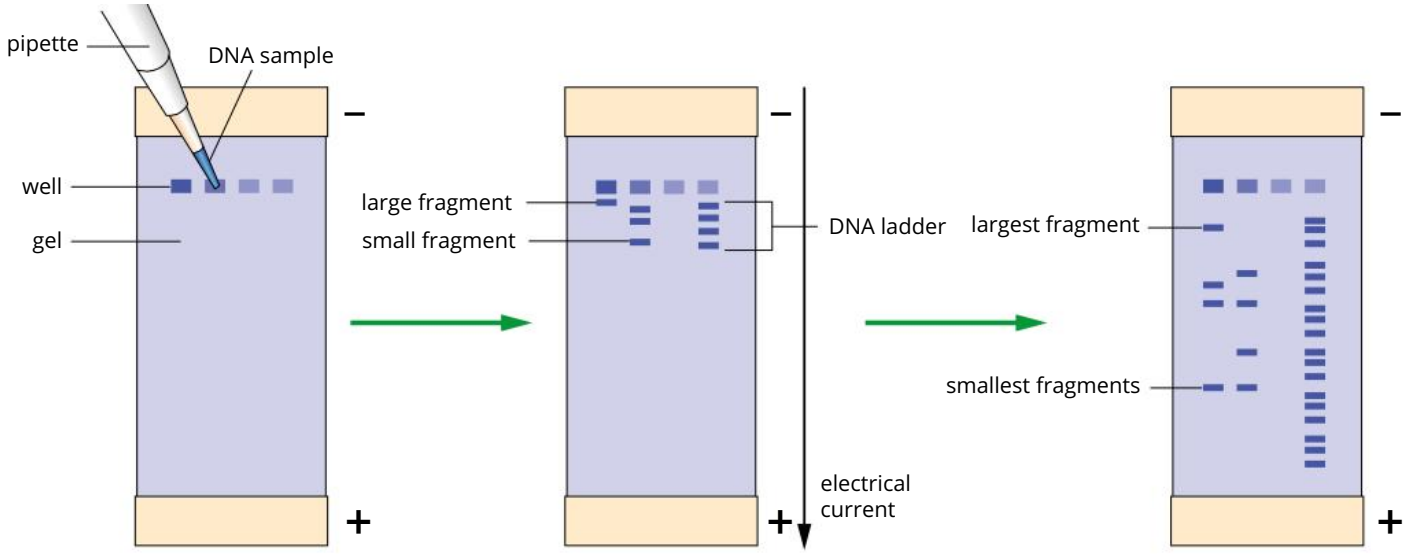


FIGURE 8.2.19 The process of gel electrophoresis, showing two DNA samples being loaded into wells and migration of the DNA fragments through the wells based on their size. The last well contains a DNA ladder for comparing and estimating the fragment length of the two DNA samples.

If the gel is stained with methylene blue, the DNA fragments are visible by eye as blue bands. If a fluorescent stain is used, the DNA in the gel is observed on a UV light box (Figure 8.2.20). This is because the fluorescent stain, which binds to the DNA in the gel, emits a bright colour when exposed to UV light. Bright coloured bands are observed wherever there is DNA present in the gel.

The size of the DNA fragment bands of various samples can be determined by comparing them against a **DNA ladder**, which contains DNA fragments of known length measured in base pairs. The DNA ladder is run on the gel next to the samples to determine the approximate length of each fragment (Figure 8.2.21).

FIGURE 8.2.20 A scientist cuts a sample from an electrophoresis gel. The gel, which was stained with a fluorescent DNA stain, shows the DNA fragments as pink fluorescent bands.

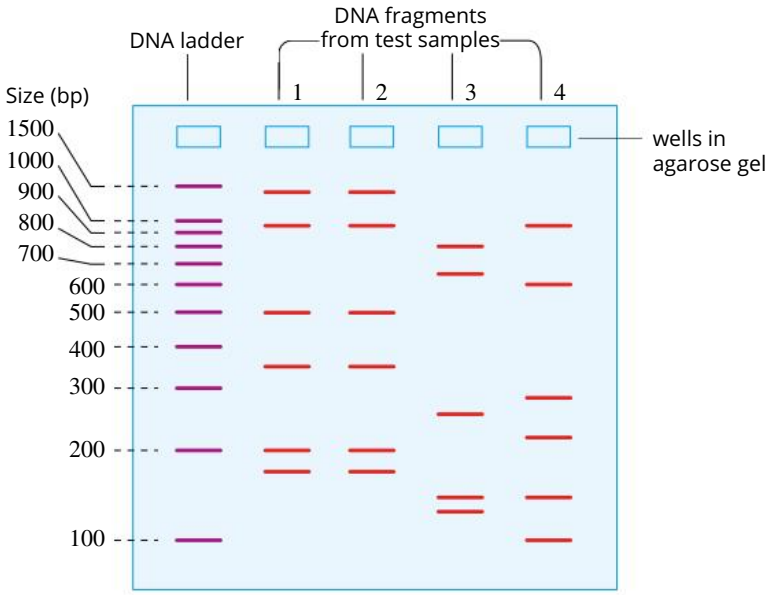


FIGURE 8.2.21 The results from gel electrophoresis can be shown as a diagram that can be analysed. Particular fragments can be identified and compared. Each fragment length can be measured in base pairs (bp) by comparing it with the DNA ladder.

BIOTECHNOLOGY SUCCESS RATES AND LIMITATIONS

When researchers promise such impressive outcomes through biotechnology, we need to know how likely they are to succeed. These techniques have a whole new meaning when applied to human life. It is important for patients to be aware of the risks or chances of success. The level of risk, whether it is temporary mild symptoms, or permanent or fatal outcomes, is important information for patients when making decisions. Also, as there is always a cost involved, patients need to know the likelihood that their financial investment will be beneficial. The following sections outline some typical experimental results illustrating the number of effective attempts to edit or change the genes of organisms.

The ratio of transformed cells to untransformed cells at the end of a transgenic experiment is the **transformation efficiency**. Transformation efficiency depends on numerous variables, such as plasmid size (small is easier), the type of DNA (supercoiled DNA or damaged DNA is harder to anneal than relaxed plasmids), the genotype of the cells, how rapidly they are growing, cell membrane structure and the method of transformation. For example, when using approximately 10^{10} viable cells for an experiment (like *E.coli* pBR322), 1 in 2000 cells may transform.

CRISPR editing in human embryos

Recent trials in China inseminated eggs that were discarded from IVF clinics with known genetically diseased sperm. The eggs were discarded from the IVF clinics when immature, then collected and matured in the laboratory. CRISPR was then used to try to repair the faulty genes in the resulting embryos.

There were two tests, one for the mutation called G1376T in the gene for the G6PD enzyme, and the other for the beta 41–42 mutation (a blood disease that causes beta-thalassemia). The results are shown in Table 8.2.3.

A mosaic is an individual composed of cells from two different genetic types.

TABLE 8.2.3 Embryonic gene editing using CRISPR

Mutation	Trials	Results
G1376T	2 embryos	1 embryo: corrected gene 1 embryo: mosaic (corrected and uncorrected gene)
beta 41–42	4 embryos	1 embryo: no correction + new mutation 1 embryo: mosaic (corrected and uncorrected gene) 2 embryos: no change

Transgenic cotton

Common biotechnology for transferring a natural pesticide gene to cotton is through the use of *Agrobacterium* (Module 8.1). Typically, very few cells will accept the genetic material delivered by the *Agrobacterium* and insert it into its own genome. If a cell accepts and inserts foreign DNA (e.g. a gene) into its genome, it is known as transgenic uptake. The transgenic uptake rate for various genetic technologies is low.

The offspring of the transgenic organisms are then used for crops, such as those seen in Figure 8.2.22.

FIGURE 8.2.22 (a) Bt cotton plants (on the left) resist insect attack due to a transgenic pesticide gene. (b) *Helicoverpa* caterpillar eating a cotton flower.

Bt cotton contributes to the 99% of total cotton crops planted in Australia employing biotechnology. GM traits such as pesticides and herbicides have been used in cotton varieties since 1996, and now up to 80% less pesticides are used.

Table 8.2.4 shows the results of one transgenic cotton plant experiment. It demonstrates that genes are not always transferred into 100% of all the cells of an organism (trial 2); however, when effective transgenic uptake has occurred (trial 1), the results can be significant.

TABLE 8.2.4 Bt cotton crop results when attacked by insects

	Number of plants without insect attack		
paddock (720 plants/paddock)	Bt cotton Trial 1	Bt cotton Trial 2	Not modified Trial 3 (control)
1 2 3	643 655 702	469 427 525	484 456 402
(mean)	666.67	473.67	447.33
1σ (std dev)	31.18	49.17	41.68

8.2 Review

SUMMARY

- DNA is often found or extracted in trace amounts and requires amplification to produce an adequate sample for scientists to work with.
- DNA amplification uses the polymerase chain reaction (PCR) to rapidly increase the amount of DNA (creating identical copies of the DNA sample).
- PCR is a technique that amplifies DNA exponentially to create millions of identical copies of the DNA from a very small sample using a three-step process:
  - denaturation
  - annealing
  - extension.
- Polymerases are enzymes that create polymers of nucleic acids (DNA and RNA). DNA polymerase acts to assemble DNA molecules, and RNA polymerase acts to assemble RNA molecules.
- Taq polymerase is a heat-resistant DNA polymerase used in PCR.
- Gel electrophoresis is a technique that separates fragments of negatively charged DNA by length.
- Restriction enzymes are enzymes that cut DNA at particular recognition sites. Sticky-end restriction enzymes leave fragments with overhanging ends that have exposed bases. Blunt-end restriction enzymes cut DNA to leave flat-ended fragments.
- Ligase enzymes permanently join fragments of DNA or RNA together in a process called ligation. DNA ligase joins DNA fragments; RNA ligase joins RNA fragments.
- Recombinant plasmids are plasmids that have had target DNA inserted into them. The same sticky-end or blunt-end restriction enzyme is used to cut both the targeted gene and the plasmid and DNA ligase is used to permanently join the two together.
- Plasmids with antibiotic resistance are generally used to enable identification of bacterial transformation later on, as only bacterial cells containing these plasmids will survive when grown in cultures containing the antibiotic.
- A gene that produces an identifiable phenotype, such as a coloured product or antibiotic resistance, may be used as a reporter gene to identify transformed cells.
- The rate of transformation is very low.

KEY QUESTIONS

Retrieval

1 **a** Explain what is meant by amplifying a piece of DNA.
b Recall what the acronym PCR represents.

2 Define the following terms and give an example of each.
a gene cloning
b recombinant DNA

3 **a** Describe the function of the enzyme reverse transcriptase.
b Explain when is it used in molecular techniques.

4 **a** Define gel electrophoresis.
b Explain what this technique reveals about the DNA fragments being tested.

5 Define a plasmid and describe the role plasmids play in gene cloning.

Comprehension

6 **a** Describe what a restriction enzyme is and its function.
b Describe the difference between sticky ends and blunt ends produced by restriction enzymes.

7 Propose two features of a plasmid that are important for identifying cells containing a recombinant plasmid.

8 Describe how antibiotics are used to select transformed bacteria.

9 Draw a simple, labelled flow diagram to summarise the key steps in the process of PCR.

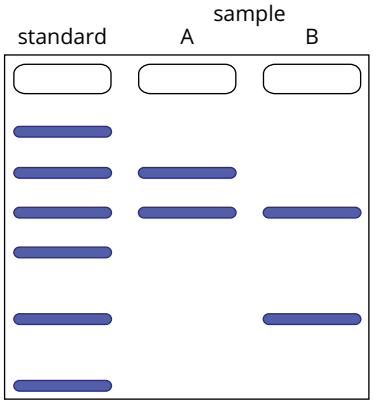
10 Outline the purpose of DNA ligase in recombinant DNA technology.

Analysis

11 Identify why Taq polymerase is used in PCR. Refer to hydrogen bonds in your response.

12 Draw a transformation flow diagram that identifies the use of the complementary strand code CTTAAG in bacterial modifications for insulin production.

13 Gel electrophoresis was conducted with a DNA ladder (standard) and two DNA samples (A and B). The diagram illustrates the resulting gel.



a Determine the direction in which DNA migrates through the gel when an electric current is applied.

b Identify the sample with the larger segments of DNA.

14 Three restriction enzymes and their recognition sites are illustrated below.

Consider the DNA sequence below.

5' AGT AGT ACT GAC GCC TAA GGA GTA CTG 3'
TCA TCA TGA CTG CGG ATT CCT CAT GAC

How many pieces would it be cut into if it was mixed with:

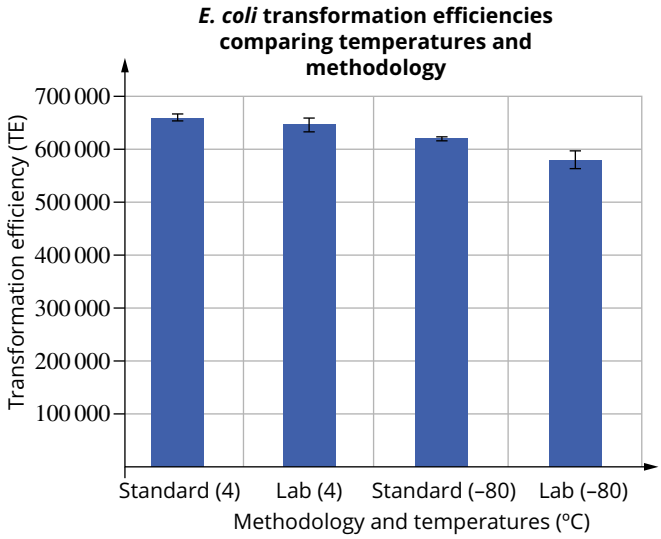
a AatII only

b AssI and AxyI

c all three restriction enzymes

15 *E. coli* was transformed using two methodologies, standard and lab protocols, and employing two different storage temperatures, 4°C and –80°C. Each bacterial sample received 0.65 ng of DNA. The transformation efficiency (number of bacterial colonies transformed per µg of DNA used) was measured. The results are shown below with the standard error represented by error bars. A comparison between the standard and lab methodologies at 4°C produced a *p*-value of 0.1951, while comparing standard and lab methodologies at –80°C produced a *p*-value of 0.0720. The transformation efficiency (TE) was calculated as follows:

$$\text{TE} = \frac{\text{number of colony forming units (CFU)}}{\text{amount of plasmid DNA (µg)}}$$



a Calculate the number of colonies transformed using the standard protocol at 4°C.

b Determine the difference in transformation efficiency between standard 4°C and lab –80°C as a percentage change from standard 4°C.

c Conclude if there was a significant difference using the *p*-values (refer to Chapter 1).

d Write a conclusion for the *E. coli* transformation investigation.

8.3 DNA sequencing

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recognise the applications of:
  - DNA sequencing to map species genomes
  - DNA profiling to identify unique genetic information
  - identifying mutations.

Sequencing DNA means determining the order of the four bases that make up the DNA molecule. The order of bases tells scientists the kind of genetic information that is carried in a particular DNA segment. For example, scientists can use sequence information to determine which sections of DNA contain genes and which areas carry regulatory instructions that turn genes on or off.

Importantly, sequence data can also highlight changes in a gene that may cause disease. DNA sequencing is being used to examine the development of common and complex diseases, such as heart disease and diabetes, and inherited diseases that cause physical malformations, developmental delay and metabolic diseases.

Comparing the genome sequences of different types of organisms can also provide details for gene mapping and classification and insights into developmental biology and evolution.

DNA SEQUENCING

Determining the order of bases for a gene is a crucial step in understanding genetics and the expression and regulation of genes. This is extremely useful for modern medicine and biotechnology in developing advanced health care. It is also being adopted by private health insurance companies in the process of profiling their clients. As outlined in Module 8.2, PCR is used to amplify DNA. However, PCR techniques can also be used to determine the sequence of a piece of DNA.

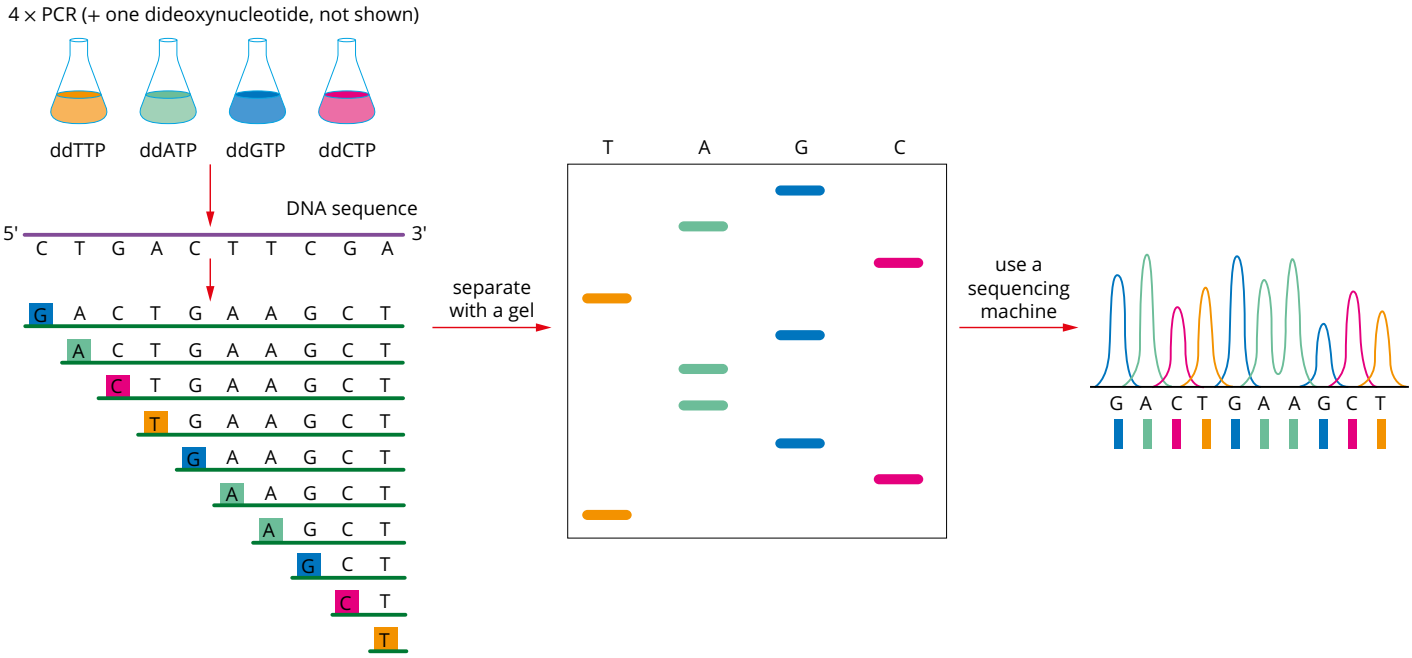


FIGURE 8.3.1 DNA sequencing for GACTGAAGCT.

To sequence a piece of DNA, a special type of nucleotide, **dideoxynucleotide**, is used. A dideoxynucleotide is missing a hydroxyl group on the nucleotide base and can be used for any nucleotide (A, T, G or C). This is the point of attachment for the next base when a strand is forming. Once added to the end of a growing strand of DNA, a dideoxynucleotide will stop the elongation and produce a shortened piece. Dideoxynucleotides are also radioactive or fluorescent, allowing scientists to distinguish between the four bases (Figure 8.3.1).

Dideoxynucleotides can be abbreviated as ddNTP (dideoxyNucleotide TriPhosphates). There are four ddNTPs:

- ddGTP (dideoxyguanosine triphosphate)
- ddATP (dideoxyadenosine triphosphate)
- ddTTP (dideoxythymidine triphosphate)
- ddCTP (dideoxycytidine triphosphate).

To sequence a piece of DNA, it undergoes PCR with a mixture of normal nucleotides and one or more known dideoxynucleotides. The presence of dideoxynucleotide in the PCR process means that the process will produce new strands of DNA of different fragment sizes. This is because when the growing strand picks up a dideoxynucleotide, the copy will terminate at that point. After millions of copies, there will be many strands with different fragment sizes (Figure 8.3.2).

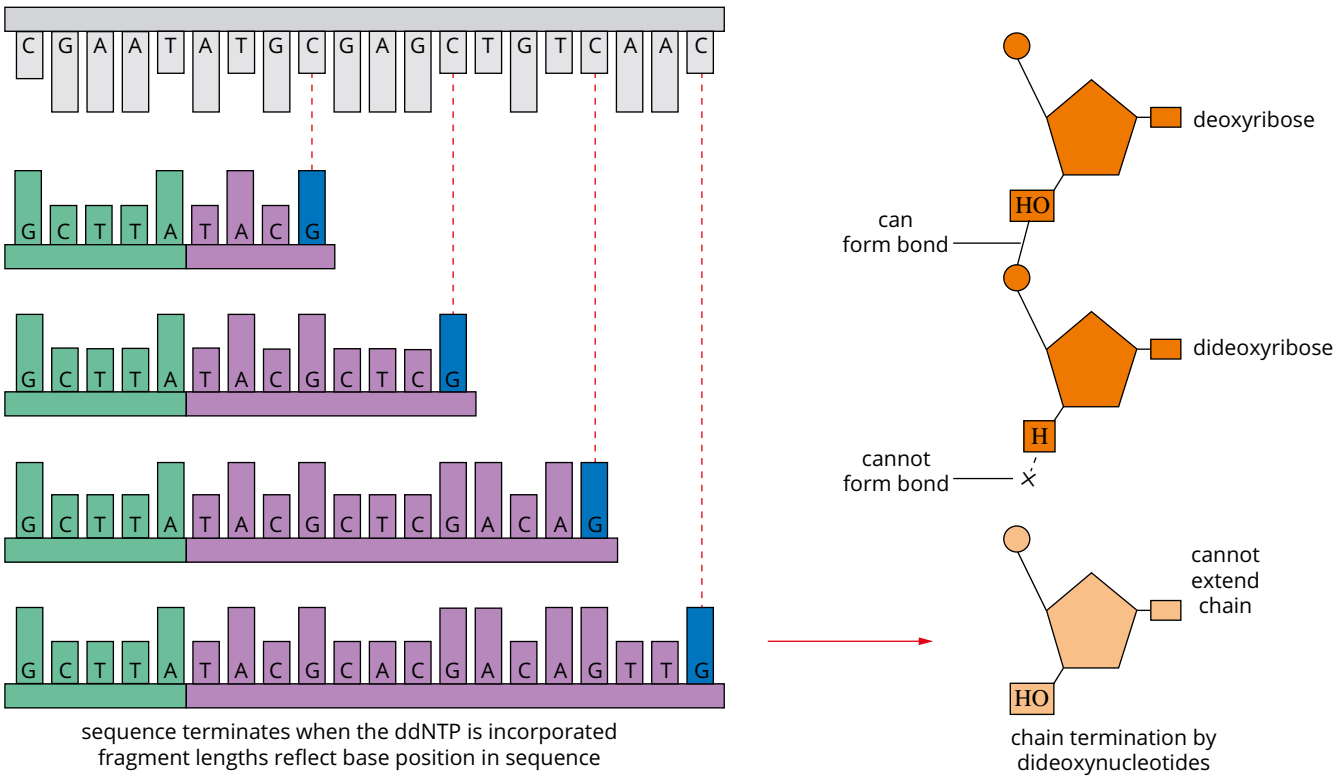


FIGURE 8.3.2 Determining nucleotide positions using dideoxynucleotides.

Gel electrophoresis (Module 8.2) is then used to separate the DNA fragments based on size. The size of the fragment length allows scientists to determine the position of a known base.

Originally, this process took an extremely long time; it took about 15 years to complete the Human Genome Project. Today, however, with advanced technology, you can have your genome sequenced in a few days.

Modern medicine and genomic studies need fast, large-scale analysis. A process called **massively parallel sequencing** is used to sequence DNA. In massively parallel sequencing, a 'shot gun' approach breaks up all the DNA into fragments and a complex computer algorithm is used to analyse all of the fragments simultaneously. This has made DNA sequencing much more affordable and practical.

These days there are also numerous methods used to break up the DNA, and chemistry to identify the sequence in massively parallel sequencing. Some examples are described in Table 8.3.1. These procedures can be used by commercial entities. Their limitations are dependent on the sensitivity of equipment, chemicals used (including the type of DNA polymerase), exposure to heat at various steps in the procedure and many others. Within each chemical procedure there are large variations in limitations and also between commercial entities.

TABLE 8.3.1 Limitations of various massively parallel sequencing procedures

Chemical procedure	Maximum length of sequence that can be read (bases)	Maximum run length (Gb)
pyrosequencing	400	0.40–0.60
reversible dye terminator	2 × 150	1000
oligonucleotide 9-mer unchained ligation	7 × 10	3000

Scientists are trying to develop a 'direct' reading of a DNA molecule without the need for messy, demanding, time-consuming reactions of DNA with enzymes, gels or chemistry. The most successful of these early technologies is **nanopore sequencing**. This method feeds a strand of DNA through a pore in a conductive material. As the bases move through the nanopore, their slightly different sizes stretch the pore, altering the pore's conductivity. The changes in conductivity can be used to determine the sequence of bases (see Figure 8.3.3).

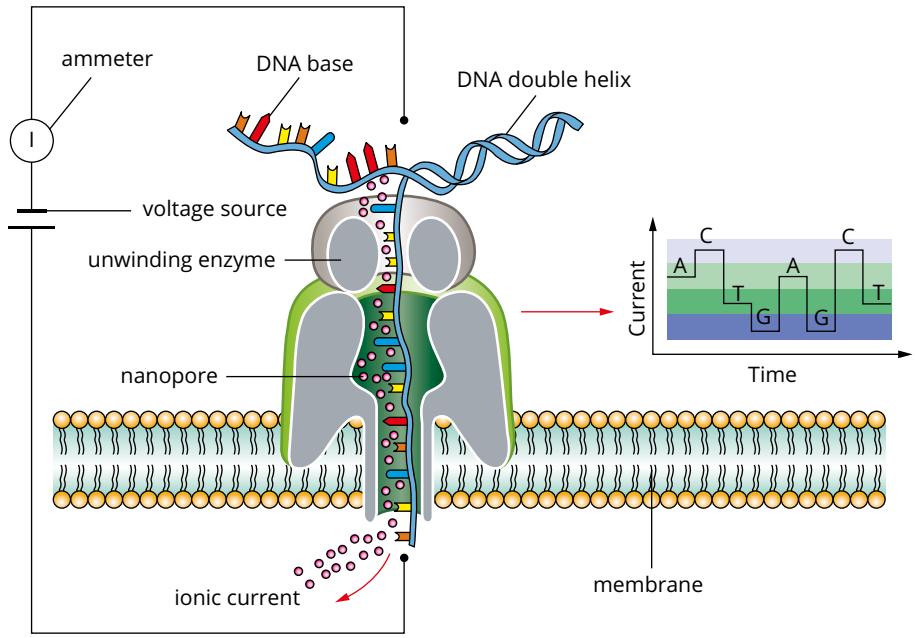


FIGURE 8.3.3 A strand of DNA is passed through a nanopore. The current is measured and translated into the DNA sequence.

The accuracy of the raw measurement is very high for most sequencing technologies (Table 8.3.2); however, sequencing technologies present challenges. They all require the use of PCR to produce enough quantities of DNA to sample, and the errors that arise during PCR carry over to the raw measurements. They also use different computer algorithms to analyse the raw data.

TABLE 8.3.2 Accuracy of raw measurements for various sequencing techniques

Sequencing technique	Raw error rate
Sanger sequencing	1×10^{-5} to 1×10^{-4}
massively parallel sequencing	1×10^{-3} to 1×10^{-2}
nanopore sequencing	2×10^{-2} to 1.8×10^{-1}

Figure 8.3.4 shows the results of a small sample of nanopore sequencing (showing 12 nucleotides from position 90 to 112 in the DNA sampled) and illustrates typical errors in all sequencing techniques. Numerous nucleotides are identified at each sequence location, as many DNA samples are measured due to amplification by PCR. The raw data (measurements) are processed by a chosen computer program that utilises algorithms to establish the most likely correct sequence based on the most common nucleotide identified for each position. Depending on the computer processor, DNA sequence run length and computer program used, it can take anywhere from four hours to 11 days to determine the DNA sequence of a sample.

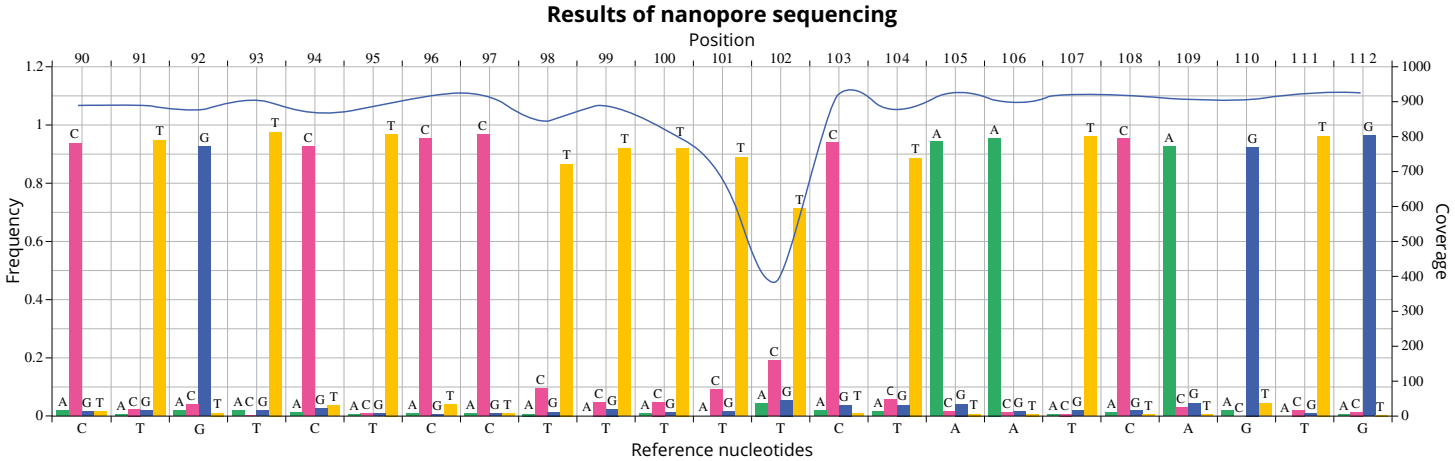


FIGURE 8.3.4 Column graph showing the nucleotides identified at each position in the DNA sequence and the frequency each nucleotide was identified. Coverage refers to the number of times that position was measured. The correct base is determined by the overwhelming frequency of a particular nucleotide, which is usually more than 90% of the raw measurement.

DNA PROFILING

DNA profiling is a technique that compares and identifies individuals based on their unique DNA sequence. Satellite DNA are long stretches of DNA made up of repeating elements called short tandem repeats (STR) found in non-protein coding regions (exons). STRs can be cut into fragments by restriction enzymes to generate unique fragment profiles. These can be visualised and analysed by gel electrophoresis. This technique can be used to determine the relatedness between species and provide a map for the complete genome, both in simple organisms and humans.

Species genome mapping

Scientists often use model organisms to understand biological processes. Model organisms are usually ones that are easy to maintain, produce large numbers of offspring and have a short generation time. They enable a large collection of data so that relationships between concepts can be analysed, interpreted and understood. Mutations in their genes can help scientists investigate the changes in a characteristic and look for patterns and predictions for human genes. Model organisms in genome mapping develop information about genes, expression, protein function and genetic regulation. Researchers can use this to investigate variants of the gene and coordination mechanisms in other species, including humans.

An example of a model organism is the nematode worm (Figure 8.3.5), which is about 1 mm long, lives in the soil and feeds on bacteria. It is small, only requires soil and has a simpler structure than many eukaryotic organisms as it has a transparent skin and no bones, heart or lungs. This means fine details can be observed quite easily under a microscope. Using a combination of biotechnologies, such as DNA sequencing techniques, PCR and gel electrophoresis, it took about a decade to map the genetic development of the worm from a single cell to a mature adult that contains about 1000 cells.

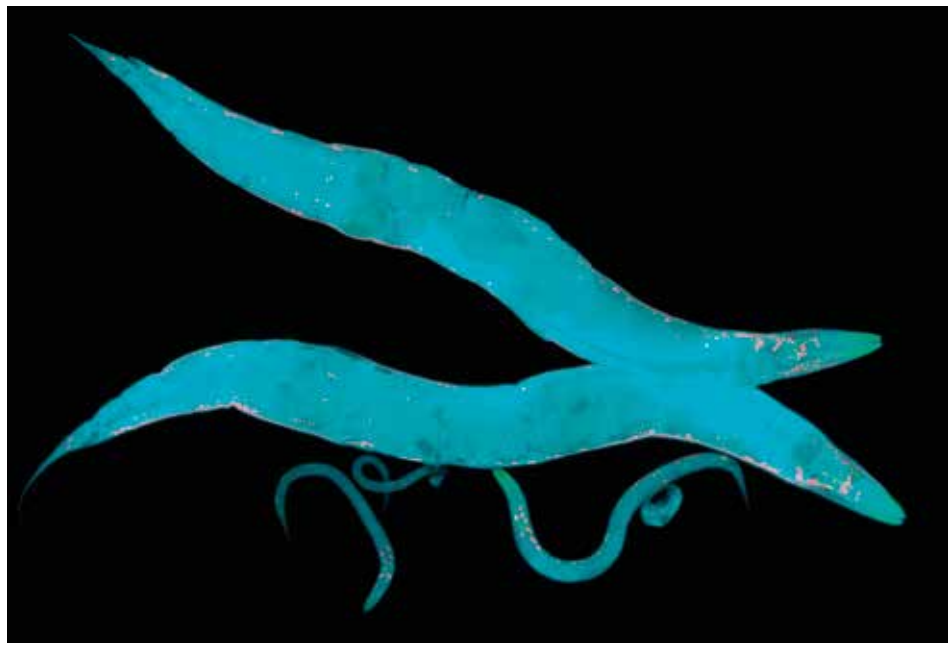


FIGURE 8.3.5 Two nematode worms stained with a fluorescent dye.

By the 1980s, a large number of mutations had been identified in the nematode worm and these genes needed to be mapped to understand what was happening at the genetic level. Using automated sequencing programs, the first 3 million bases were sequenced. This was only 3% of the whole genome, but it showed that with this technology it would be possible to map the human genome.

In 1988, the nematode worm was the first animal to have its full genome published. It was found to have 20 500 genes. The genetic sequences are contained in a database called Wormbase, a central resource that is constantly updated. The data recorded is highly detailed and includes genetic maps of several nematode species, information on various mutants and their characteristics and details about the worm's nervous system. This has allowed whole gene families to be identified and patterns of gene expression, like **programmed cell death** to be understood.

Scientists are searching for and mapping genes related to ageing (genes have been identified in the worm that limit lifespan), cancers, **apoptosis** (cell death that occurs as a normal and controlled part of an organism's growth or development) and new treatments for Alzheimer's disease.

Human Genome Project

The objectives of the Human Genome Project were to:

- provide a complete and accurate sequence of the 3 billion DNA base pairs that make up the human genome
- find all of the estimated 20 000–25 000 human genes.

Essentially, the blueprint provides information about the structure and function of genes, and the proteins they produce and regulate. (The genomes of several other organisms have been sequenced as well, for example, mouse, yeast, roundworm and fruit fly.)

The main goal of the Human Genome Project was to produce a high-quality DNA sequence that could serve as a common reference for understanding the genetic basis of health and disease. A public catalogue of human variation and genotype data was run between 2008 and 2015. It is called the 1000 Genomes Project and has provided reference data for genetic variants with frequencies of at least 1% in the populations studied (polymorphisms). With the speed and volume of sequencing technology like BLAST, most variants can be identified.

Three pilot studies were initially undertaken to guide the strategies of the Human Genome Project.

- Pilot 1 looked at whole genome sequencing of 180 samples, 2–4 times each (low coverage).
- Pilot 2 investigated whole genome sequencing of two mother–father–child trios, with 20–40 repeats.
- Pilot 3 investigated 1000 gene regions in 900 samples at least 50 times.

The first pilot study was done to determine a strategy of sharing data across the samples and had a quite low coverage. Coverage means the number of times a sequence of DNA was read or underwent raw measurement. This ensured the methods were suitable and that researchers did not waste too much time repeating the tests.

The trio tests for the second pilot study picked up the relatedness of the gene sequences and determined the level of coverage needed to pick up different variants.

The third pilot was used to fine tune the methods for gene-region capture before the main study. Figure 8.3.6 shows the samples for the 1000 genome project.

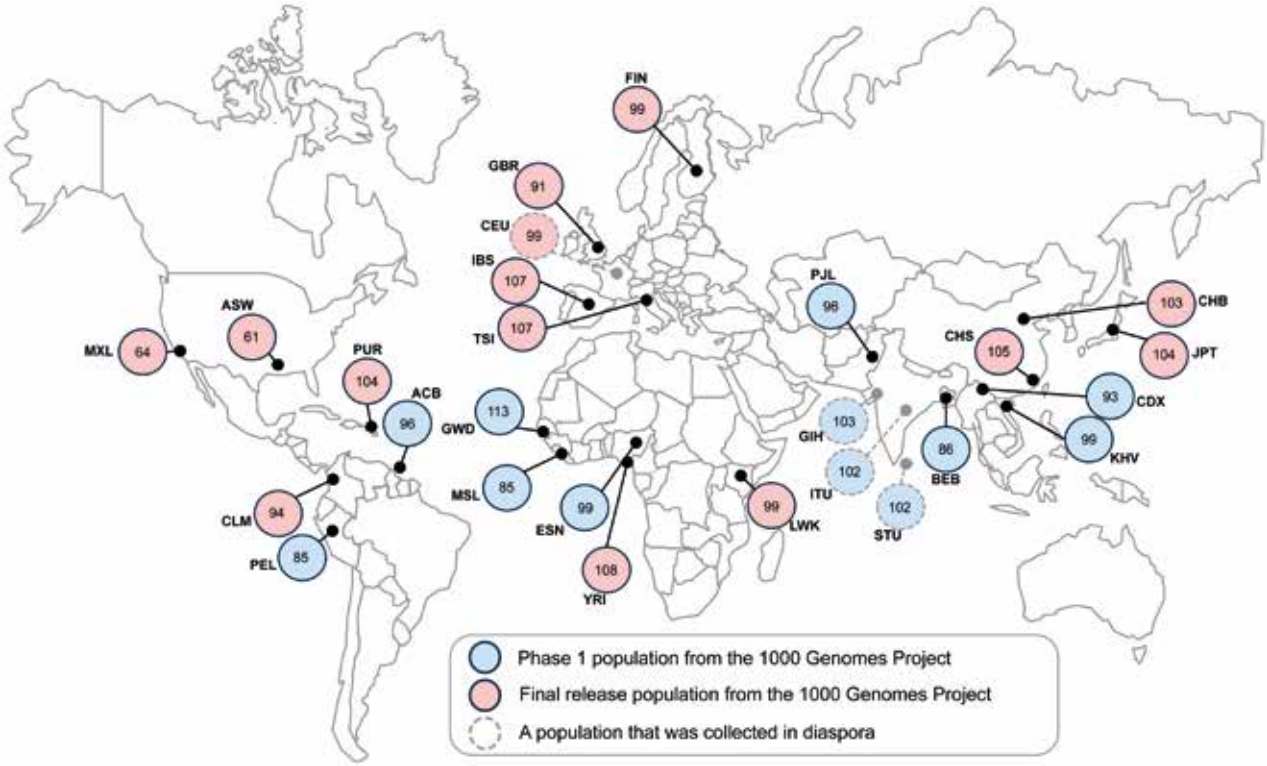


FIGURE 8.3.6 Sample location and sizes used in the 1000 Genomes Project. Each circle represents the number of sequences released.

The final study contained data from 2504 individuals from 26 different populations, with 24 individuals completely sequenced with high coverage. Both the pilot and final studies were published. The samples are completely anonymous, with no medical or phenotypic data. Individuals had to complete consent document to ensure they were aware that the data would be made public. Ethical guidelines prevented their names, ethnicity or gender from being released.

The results have shown that there is a higher level of variation in the human genome than the Human Genome Project first indicated. The current human population is over 7 billion and growing. It is assumed that new mutations are accumulating at the rate of between 1 and 100 mutations per generation, based on models and limited empirical data. The current reference sequence has been based on a limited number of samples and it does not adequately represent the full range of human diversity, nor is it complete. However, it is a starting point.

Ethical, legal and social implications were considered as part of the Human Genome Project. These included:

- privacy and fairness of collected genetic profiles to avoid discrimination for employment and insurance
- getting patients to accept new genetic technologies and screening as part of normal medical practice
- ethical issues with informed consent by participants in any of the studies
- education of the public about genetics and the complex issues that result from this type of research
- accuracy of the results.

WS 4.1.10

DETECTING MUTATIONS

The difficulty with detecting mutations depends on the location of the mutation and whether or not established knowledge exists. As shown earlier, some DNA sequencing technology is highly accurate, with an error rate between 1×10^{-4} to 1×10^{-5}. Detecting the DNA sequence is achievable. The challenge is identifying mutations within the sequence.

Mutations that cause genetic disorders are often obvious as there are visible symptoms. These mutations typically occur in the coding regions of the genetic code, although some remain undetected until symptoms become visible.

Some mutations in the coding regions have been catalogued by genetic databases such as Online Mendelian Inheritance in Man and ClinVar. This allows DNA sequences to be compared to the genetic databases and mutations to be identified. Hundreds of thousands of mutations have been identified and catalogued; however, most genetic research until recently has focused on the approximate 2% of the genome that is coded. Although the genes loci of the human genome has been mapped, there is still significant research to be done, given there are many polymorphisms and alleles yet to be identified. Further, detecting mutations in unknown regions of code is an extremely difficult task (Figure 8.3.7).

It is essential that an accurate analysis is made, because some mutations may involve only one base pair. These can be detected with **molecular methods** like direct sequencing, DNA hybridisation or restriction enzyme digestion methods. The methods used will depend on whether the mutation is one that is already identified or a completely new variation.

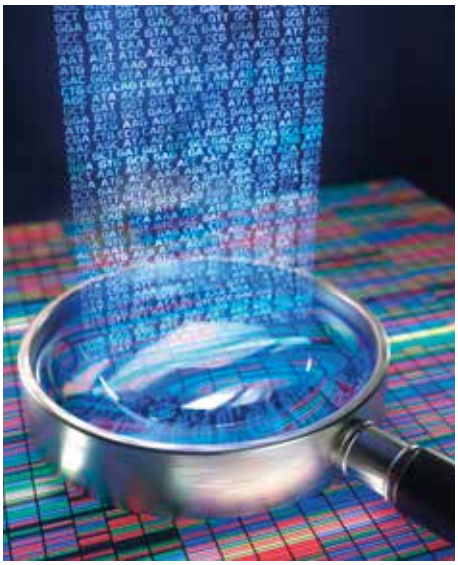


FIGURE 8.3.7 Although gene loci have been determined in the human genome, allelic variation and other polymorphisms are still to be researched.

Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) is a refined and specific PCR technique. MLPA is a PCR technology that can detect single nucleotide mutations. MLPA works as it selectively amplifies a genetic sequence that contains a mutation. It uses synthesised DNA sequences, known as probe oligonucleotides. The probe oligonucleotides contain a single nucleotide variation to a healthy sequence, and therefore target a single mutation to amplify. The probe oligonucleotides also have fluorescent tags, so they can be seen in gel electrophoresis. They also have other sequence regions that encourage and enable their synthesis during PCR.

The probe oligonucleotides for MLPA consist of two pieces (Figure 8.3.8). The two pieces will only ligase if the complementary sequence is found in a sample. Consequently, the probe oligonucleotides only ligase if the sample DNA contains the single point mutation that matches the probe. As mentioned, this PCR technique only amplifies DNA sequences with a mutation. Therefore the gel electrophoresis will only show results from mutations.

When MLPA is used, several probe oligonucleotides are used. This means that multiple single nucleotide variations and their locations can be identified in the DNA sequence. This allows the type of mutation and its location to be determined.

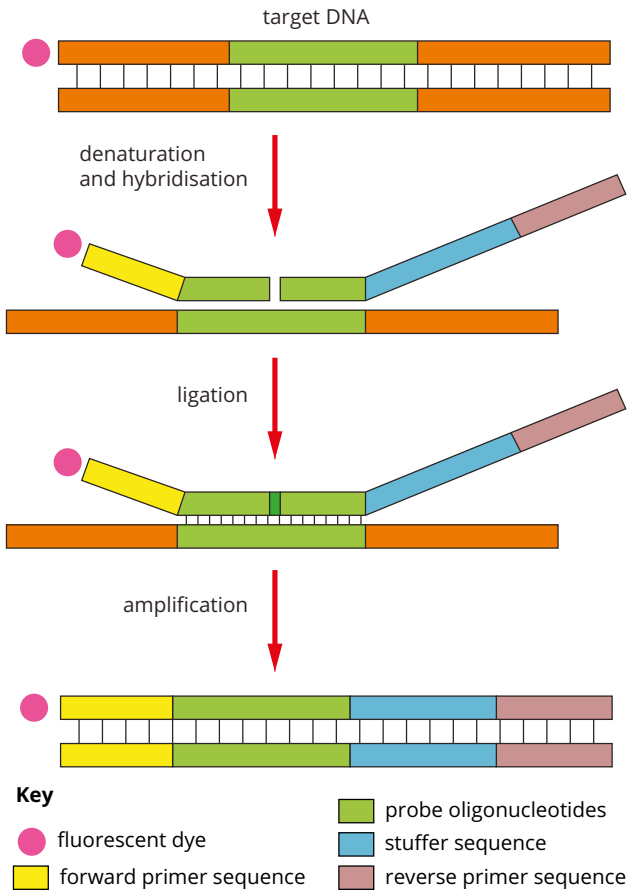


FIGURE 8.3.8 A schematic summary of the MLPA technique. The probe oligonucleotides are synthesised in two pieces. These two pieces of probe oligonucleotides will only ligase if the sample DNA sequence contains the same sequence, including the single nucleotide variant (mutation). PCR will amplify the DNA sequence with the mutation and the corresponding fluorescent tag.

Duchenne muscular dystrophy (DMD) is a genetic disease caused by a mutation in the sequence of the dystrophin gene, which is located on the X chromosome. MLPA has been used to detect many polymorphisms of this gene and numerous mutations. The dystrophin protein keeps the muscle fibres intact and also protects them. By about the age of three, affected children show progressive muscle weakness and by adolescence the heart and respiratory muscles are affected. The normal gene is 2 220 390 base pairs long and contains many exons and introns. The total length of the coding sequences (exons) is 11 055 nucleotides.

Over 2000 DMD mutations have been found so far. The position refers to the amino acid sequence where the mutation occurs; the description refers to the mutation that occurred, such as changing the amino acid leucine to arginine; the graphical view shows the position along the DNA sequence of where the mutation is found in a schematic diagram; and the length is the number of amino acids affected.

Some of the limitations of MLPA are that it produces inconclusive results in 1 out of 4 tests. There are numerous reasons for this, including insufficient quantity of sample DNA and unsuccessful MLPA reactions due to poor sample DNA quality (such as degradation of the sample or nuclease contamination). MLPA is highly susceptible to contaminants.

Another limitation is that the sample DNA for the probe oligonucleotides to use as a template must be more than 50% of the sample to achieve valid results. This can be challenging in some instances. For example, when detecting the mutation in cancer cells, more than 50% of the sample DNA must come from the cancer cells. This is difficult, as biopsies often contain both cancerous and non-cancerous cells.

PA 4.1.5

Circle sequencing

Many DNA sequencing technologies amplify a linear segment of DNA. Theoretically, this would produce numerous copies of the same sequence, and when amplifying numerous DNA samples, a cross comparison can determine variants (polymorphisms and mutations). However, errors occur during the PCR process and also during the raw reading process, both causing an increase in variants. Circle sequencing is a technique that reduces the number of errors.

In typical DNA sequencing of linear DNA samples, barcodes are added to each DNA sample so that they can be identified later as separate DNA samples. Once the barcodes have been added to each DNA sample, PCR amplifies them all. Many errors occur; however, a computer program is used to group all of the barcoded DNA samples together into families. The DNA is then analysed to determine the DNA sequence. One issue with this process is that not all barcoded DNA samples are amplified equivalently. This means that the effect of errors is not equally distributed across the DNA, which reduces the reliability of the data.

The process of circle sequencing is illustrated in Figure 8.3.9. Circle sequencing copies the DNA sample and creates a circular strand. This is done with all DNA samples. Each DNA sample is amplified; however, the circular DNA will be synthesised approximately four times per amplification, creating a long DNA strand with a sequence that is repeated four times (tandem repeats). Although errors occur, there are multiple DNA strands to analyse. A computer algorithm is used to synthesise the data to identify and remove these errors. Because the sample size is controlled and the analysis can remove more errors in the raw data, the sequence produced is more accurate.

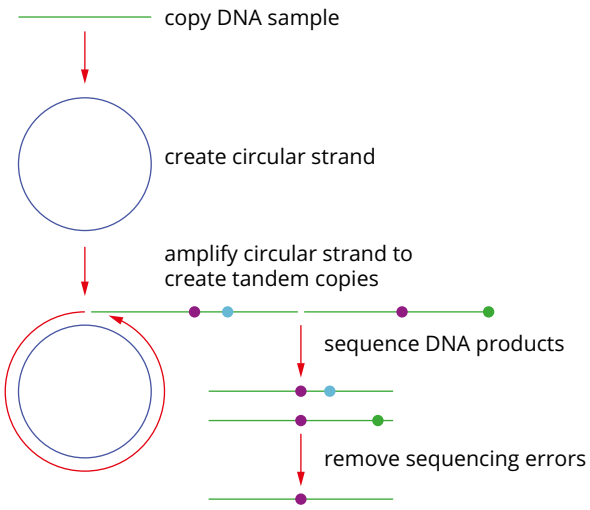


FIGURE 8.3.9 The circle sequencing process. The purple and green dots represent amplification errors. The purple dot represents a natural mutation.

Some techniques of circle sequencing have achieved error rates of 7.6×10^{-6} bases. Limitations still exist, such as sensitivity of equipment, chemicals used and exposure to heat. Unique to circle sequencing, research strongly suggests that the library preparation step (creation of the circular strand) could possibly cause the majority of errors in this technique.

8.3 Review

SUMMARY

- DNA sequencing techniques tag the nucleotide bases (ddGTP, ddATP, ddTTP, ddCTP) so their order can be found along a section of DNA.
- This process uses computer algorithms to analyse the banding patterns and sequence the base order in a gene.
- Once the sequences are known, similarities can be used to determine variants in genes (polymorphisms and mutations) and map species genomes.
- Amino acid sequences can be inferred from the DNA data, but direct sequencing is the preferred method as proteins often undergo post-transcriptional modifications.
- The complete human genome has been sequenced and this has led to a better understanding of genes, disease diagnosis and treatment. It has also raised ethical and legal questions over the rights to the information.
- Model organisms can be used to investigate the mechanisms that may work in human genetic systems, such as programmed cell death.
- Mutations are detected using PCR and gel electrophoresis.
- Mutations may directly change the protein (in the coding regions of DNA) or change the regulation of the gene (in the non-coding regions of DNA).
- The identification of mutations can be used by doctors to provide advanced health care. It is also being adopted by private health insurance companies.

KEY QUESTIONS

Retrieval

1 State the purpose of genome mapping.

2 Describe why model organisms are used in biological research.

3 Describe a benefit of sequencing DNA.

Comprehension

4 Explain the purpose of computer programs with algorithms to process the raw measurements of DNA sequencing.

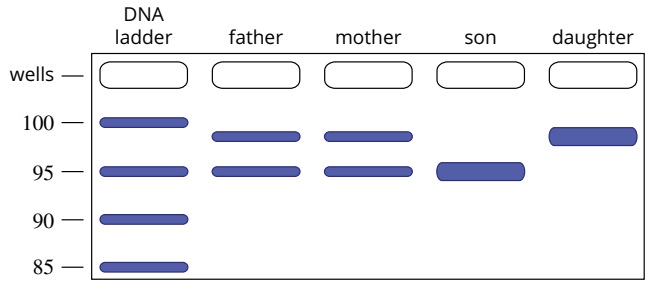
5 Describe the result of a PCR error in DNA synthesis approximately halfway through the process.

Analysis

6 Calculate the number of errors, as a range, that could occur from using the Sanger sequencing technique (Table 8.3.2 on page 417) when 1620 Gb are analysed.

7 Determine the difference in the percentage error rate between Sanger sequencing and massively parallel sequencing.

8 Members of a family with a history of cystic fibrosis underwent genetic testing to determine whether they carried the common ΔF508 mutation. DNA samples obtained from cheek cells were analysed by PCR using primers specific for the ΔF508 region, followed by gel electrophoresis. The normal allele yields a 98 bp DNA fragment. The mutant allele yields a 95 bp DNA fragment.



a Determine the genotype for the parents.

b Determine the genotype for the son and daughter.

9 Research conducted in 2010 claimed to have successfully applied DNA sequencing of exomes to discover the gene for Miller syndrome. A summary of the research includes:

- massive parallel sequencing employing 40× coverage
- Sanger sequencing was used to confirm massive parallel sequencing
- the raw sequence measurements were analysed using two different computer programs
- non-synonymous SNPs were accounted for using National Center for Biotechnology Information and University of California Santa Cruz databases
- the DNA samples were collected from four Miller syndrome patients. Two were siblings, but they were unrelated to the other patients who were from unrelated families.

Argue the reliability and validity of this research.

Chapter review

WS 4.1.12 WS 4.1.13

08

KEY TERMS

anneal
apoptosis
autism
bacterial transformation
bacteriophage
Basic Local Alignment Search Tool (BLAST)
biofilm
bioinformatics
bioweapon
blunt-end restriction enzyme
Cas protein
competence
complementary DNA (cDNA)
CRISPR
dideoxynucleotide
DNA amplification
DNA ladder
DNA ligase
DNA profiling
DNA sequencing
DNA thermocycler
electroporation
gel electrophoresis
gene cloning
gene editing
gene therapy
genetic modification
genetically modified organism (GMO)
germline
guide RNA (gRNA)
Human Genome Project
human microbiome
lacZ gene
ligase
ligation
lyse
macromolecule
massively parallel sequencing
microinjection
microsatellite
molecular method
nanopore sequencing
palindrome
patent right
polymerase
polymerase chain reaction
polymorphism
predisposition
primer
programmed cell death
pronucleus
recognition site
recombinant DNA
recombinant plasmid
restriction enzyme
reverse transcriptase
RNA ligase
sticky-end restriction enzyme
Taq polymerase
thermophilic
transfection
transformation efficiency
transformed
transgene
transgenic organism
transposon
virulence
xenotransplantation

KEY QUESTIONS

Retrieval

1 Determine which of the following is correct.
- **A** The amino acid sequence determines the order of bases on DNA.
- **B** A single change in the DNA will always cause a change in the amino acid sequence.
- **C** A single base change in the DNA may not alter the amino acid sequence in the protein produced.
- **D** The number of differences in the amino acid sequence of a polypeptide would be identical to the number of differences in the DNA sequence of the coding gene.

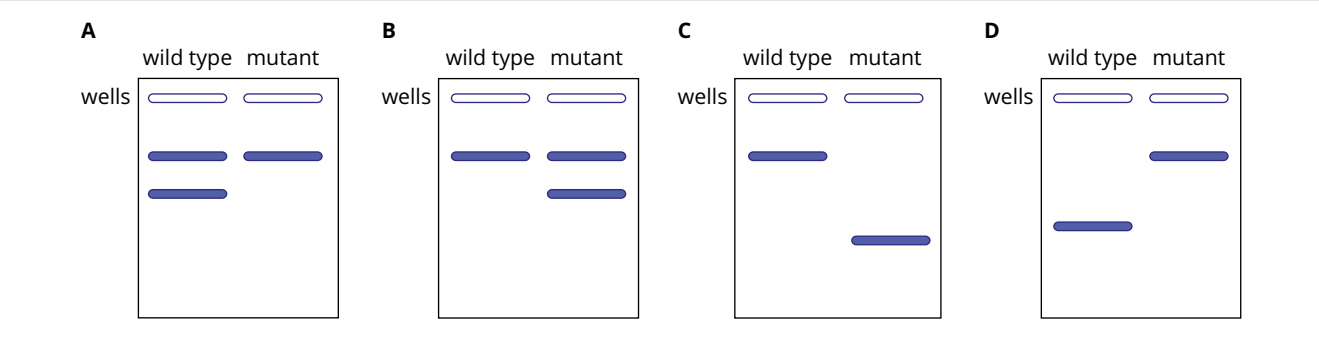
2 Identify which of the following would be a suitable target DNA for PCR.
- **A** a genome
- **B** a microsatellite
- **C** a whole chromosome
- **D** the nuclear membrane

3 Indicate which step in the process of PCR best describes annealing.
- **A** binding the primers
- **B** adding the polymerase
- **C** separating the DNA strands
- **D** building the complementary DNA strands

4 Choose the correct statement about gel electrophoresis.
- **A** it matches DNA sequences
- **B** it separates the genes into separate bands
- **C** it separates DNA fragments according to size
- **D** it draws the fragments of DNA from the positive to the negative electrode

Comprehension

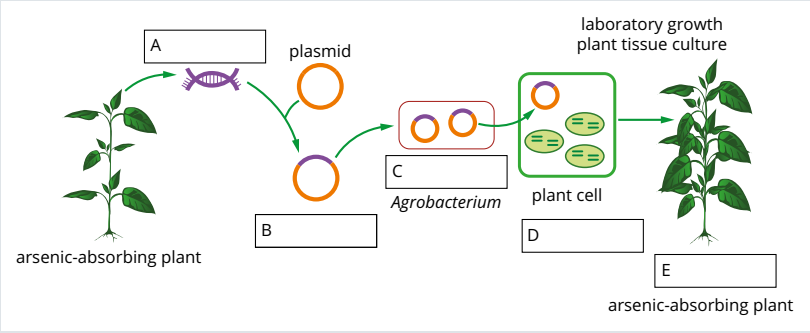
5 *Drosophila melanogaster* is commonly used for genetic research. One particular mutation results in the deletion of a section of DNA 200 bp long from one particular gene. The gene was extracted from a fly that is homozygous for the mutant gene and the same gene was extracted from a fly that is homozygous for the wild type (normal) version of the gene. Both versions of the gene were amplified using PCR and then run through gel electrophoresis. The target site for the restriction enzyme used in electrophoresis is found in the 200 bp section lost in the mutation. Determine which gel most accurately shows the PCR products.



6 Tomatoes naturally have a short shelf life due to the effects of the enzyme polygalacturonase. This enzyme catalyses the breakdown of the cell walls of the tomato, causing the tomatoes to become soft and unappetising. To slow down this process, the sequence of the polygalacturonase gene was determined and an antisense gene was produced. The antisense gene has a complementary nucleotide sequence to the polygalacturonase gene. The antisense gene was inserted into the tomatoes. When the antisense gene is transcribed, the mRNA produced is complementary to the mRNA for the polygalacturonase gene, so the two mRNAs join to form double-stranded mRNA. Double-stranded mRNA cannot be translated, so the enzyme is not formed and the cell walls are not broken down. The Flavr Savr tomatoes can be considered to be:

A organic
B only transgenic
C only genetically modified
D both genetically modified and transgenic

7 Arsenic contamination of soil is a serious problem in some countries. The arsenic contaminates groundwater and drinking wells. The flow chart at right illustrates a process used to insert a gene that enables plants to absorb arsenic from the soil. Name the objects that are labelled A–E.



Analysis

8 Bacteria are commonly genetically engineered to produce human proteins. The DNA sequence for the gene of one of these proteins is shown below.

```
                                                                  ↓
5' – GCT  TCT  TCC  CGT  GCA  TAT  AGA  TAC  TCT  GAA  ACA  CTG  TGC  GGC  GGT  GAA  CTG
3' – CGA  AGA  AGG  GCA  CGT  ATA  TCT  ATG  AGA  CTT  TGT  GAC  ACG  CCG  CCA  CTT  GAC

        10 000 bp   CTG  TGC  ACC  TAT  TGT  GCT  ACT  CCC  GCA  AAG  TCC  GAA  TAG  TAG  GCT  TCT
                    GAC  ACG  TGA  ATA  ACA  CGA  TGA  GGG  CGT  TTC  AGG  CTT  ATC  ATC  CGA  AGA

CGC  TGC  TCC  CGT  GCT  TCT  CGC  GTA  TGT  CCG – 3'
GCG  ACG  AGG  GCA  CGA  AGA  GCG  CAT  ACA  GGC – 5'
```

A restriction enzyme is used to cut the gene from the human genome. Four possible enzymes have recognition sequences and cutting sites as shown.

GC|TTCT	C|TGTGC	TCC|CGT	CTG|TGC
CGAA|GA	GACAC|G	AGG|GCA	GAC|ACG
enzyme 1	enzyme 2	enzyme 3	enzyme 4

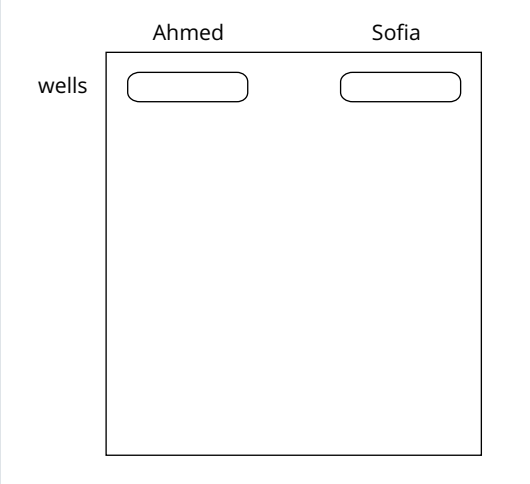
The DNA sequence is quite long, so only the beginning and end are shown along with a section before and after the gene. The start and stop triplets are underlined.

a Explain which of the restriction enzymes would be most suitable to cut out the gene so that it can be inserted into the bacterium that will produce the protein.

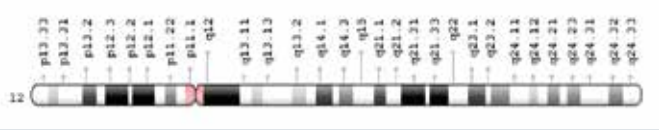
b A mutation can occur that changes the base indicated with the arrow from T to C. One way to identify individuals who have this mutation is to cut the DNA with a restriction enzyme and run the DNA on an electrophoresis gel. Explain why enzyme 4, rather than enzyme 2, is the most appropriate to use for this purpose.

c This mutation runs in one particular family. Ahmed and Sofia are members of the family and decide to be tested. Ahmed turns out to be normal and Sofia is heterozygous (one normal and one mutant allele). Assume that the entire length of the DNA section under analysis is 10 129 bp long.

- **i** If enzyme 4 is used, predict how many DNA bands will result from the cutting of Ahmed's DNA.
- **ii** If enzyme 4 is used, predict how many DNA bands will result from the cutting of Sofia's DNA.
- **iii** Re-draw and complete the picture of the electrophoresis of the DNA of Ahmed and Sofia after cutting with enzyme 4.
- **iv** Show the positions of the positive and negative terminals on your copy of the electrophoresis set below, and explain why you placed them in those positions.



9 Geneticists use diagrams called ideograms as a standard representation for chromosomes. Each section (black, grey or white) is a numbered region where identified genes are found. Ideograms show a chromosome's relative size and its banding pattern, which is the characteristic pattern of dark and light bands (dark bands being rich in A–T pairings). These bands are used to describe the location of genes on each chromosome.



It is estimated that chromosome 12 has 1100–1200 genes that provide instructions for making proteins.

a Discuss why the estimated number of genes varies.

b True or false: p12.3 indicates a larger gene than p13.2. Write a justification for your response.

10 Strawberries are a very fragile fruit. If they are exposed to freezing temperatures, the fruit becomes soft and unappealing. This results in considerable economic loss to strawberry farmers. Scientists have been searching for a way to make strawberries more resistant to frost. One approach that is still in laboratory trials is to genetically engineer strawberries. Arctic flounder live in near-freezing waters but their blood does not freeze because they make a protein that acts as an antifreeze. Scientists have cut this gene from the genome of the Arctic flounder and inserted it into a plasmid. They have also inserted into the plasmid a gene that makes epidermal cells produce a blue pigment.

Agrobacterium tumefaciens is used to modify the strawberry cells. When the strawberry cells have been successfully modified they produce both the antifreeze protein and the blue pigment. Blue frost-resistant strawberries are produced.

a Discuss whether the genetically modified strawberries are transgenic.

b Describe the steps needed to make the strawberries frost-resistant.

---

11 The 'Ever-Open Convenience Store' had experienced a number of robberies. The police were keen to catch the offender, who brandished a gun during each robbery. The police had five suspects, but were unable to gather sufficient evidence to clearly identify the perpetrator. The robber wore rubber gloves, a mask, concealing clothing and a balaclava. After the fourth robbery, the police found the little finger ripped from a pair of rubber gloves. The piece of glove was carefully collected and sent to the forensic science laboratory to be tested for DNA. Such material will contain a very small amount of DNA, if any.

a Determine a likely source of the DNA that could be found inside the glove.

b Explain the process the forensic scientists will employ to acquire enough DNA to create a DNA profile.

c A DNA profile was made using five suspects. The profile is shown here below.

i The standards are 1000 kb, 2000 kb, 4000 kb, 5000 kb, 7000 bp and 10 000 bp. Calculate the size of the DNA in the bands for suspect 5.

ii Explain which suspect best matches the crime scene sample.

iii Write an argument that concludes whether or not the perpetrator of the robbery has been identified.

12 Myotonic dystrophy is a serious disease that causes wastage of muscles. It can affect cardiac muscle, resulting in heart problems. The most severe form of the disease is caused by a mutation in the *DMPK* gene, which is found on the q arm of chromosome 19. It is caused by a CTG trinucleotide repeat. In most people there are 5–37 repeats but in individuals with myotonic dystrophy the number of repeats exceeds 50. It is often an adult-onset disease and has an autosomal dominant pattern of inheritance. This means that if the allele is inherited it is certain that the disease will develop, but the person may not know until later in life.

a Explain how electrophoresis could be used to identify whether an individual has the mutated allele.

b Outline some considerations a genetic counsellor should be cautious of when sharing positive results for myotonic dystrophy to a healthy adult.

---

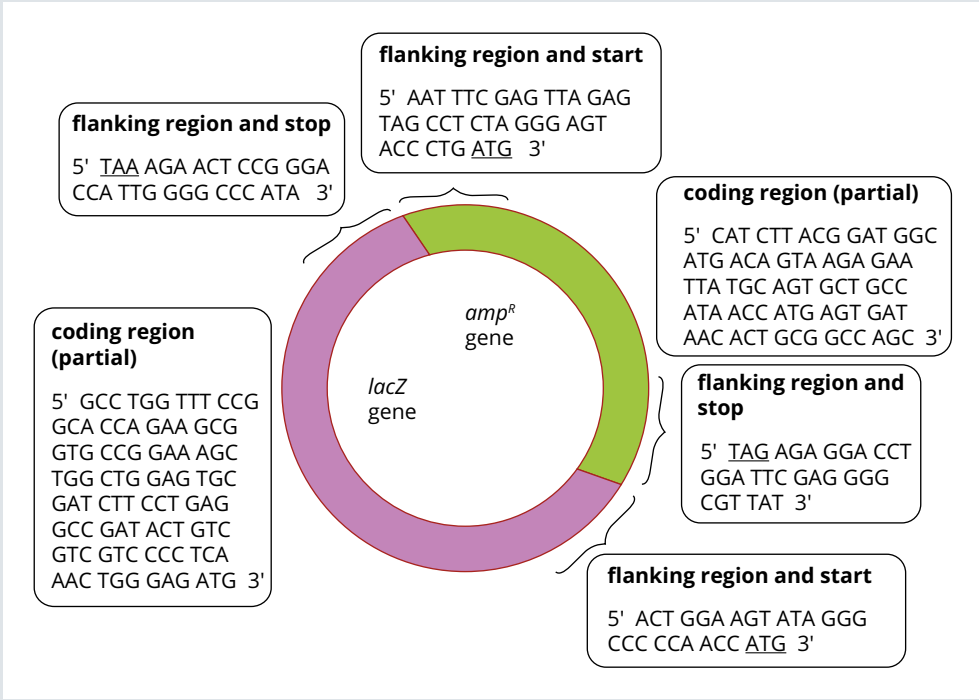
13 Scientists are testing a mutated protein that has been shown to protect against Kuru disease. They need to insert it into a plasmid for mass production. The diagram below shows the coding sequences of a few genes in the plasmid which the gene for the potentially protective protein will be inserted between.

The potentially Kuru-protective protein gene will be inserted between the *lacZ* flanking and stop sequence and the *amp*^{R} flanking region and start sequence.

In the table below are four restriction enzymes you could use. The enzymes have cutting sites shown by a slash (/). Enzymes 1 and 2 create sticky ends and enzymes 3 and 4 create blunt ends. Conclude which restriction enzyme is required.

Possible restriction enzymes for Kuru protection protein gene

Enzyme	1	2	3	4
Cutting site	ATA/AAT	GGG/TAT	ATA/TGA	TAT/TTA



14 Severe Combined Immune Deficiency (SCID) is an inherited disease. The most common form of the disease has been linked to mutations in the gene *IL2RG*, which is found on the X chromosome, so this form of the disease is called SCID-X1. As a result of the mutation, the body lacks the ability to make natural killer cells, T cells and B cells, effectively resulting in a total absence of the adaptive immune response and a depleted innate response to viruses. Typically, individuals die from viral infections within the first year of life. Its inheritance is X-linked recessive.

a **i** Discuss how scientists can learn to distinguish the disease mutations from the *IL2RG* polymorphisms. Discuss only the samples required, technology/technique employed and reliability of the results.

ii Explain how a scientist would non-invasively identify an infant with SCID using genetics rather than DNA sequencing.

b A gene therapy trial attempted to correct the gene in 20 boys from 1999 to 2002. Bone marrow stem cells were collected from the patients and the active form of *IL2RG* gene was inserted into the nuclei of the cells using a viral vector. Eighteen boys developed the ability to make effective lymphocytes. Long-term monitoring showed that five of the boys developed leukaemia and, of those, one eventually died. Further research into the causes of the leukaemia discovered that the cancer developed because the *IL2RG* gene inserted into the chromosomes by the virus was inserted in such a way as to activate a cancer-causing gene.
Argue how the insertion of a virus might cause a cancer-causing gene to become activated.

15 A 1200 base pair (bp) DNA sequence of cytochrome *b* for several species of lizard were analysed. To accomplish this, restriction enzymes were used to cleave the DNA then gel electrophoresis with radioisotope tagging. The results are shown in the table below showing the number of amino base differences.

a In the table, large numbers imply that pairs of populations are less related. Explain the reason.

b Identify which two populations are most closely related. Justify your answer.

c Identify the organisms that are least related to the rest. Explain your reasoning.

d Draw the relationship between the populations as a tree, using the differences in cytochrome *b* as your guide.

e The researchers also used other techniques such as geographical distance, distribution and body structure to build phylogenetic trees.

i List three reasons why techniques comparing DNA sequences may be considered more reliable than techniques comparing geographical distance, distribution and body structure.

ii The length of the DNA sequenced was 1200 base pairs. Discuss what factors may have introduced sequencing errors that were not mutations.

Knowledge utilisation

16 You are going to find genes in a GenBank by name.
To go to the National Center for Biotechnology Information website, search for 'NCBI' using an internet search engine.
In the search box, type 'nucleotide'. From the search results, select web resource 'nucelotide'. In the new search box, type the gene name and species you want to search. Try 'insulin human' to start. You will find a long list of published sequence data. Find the entry that has the sequence for the complete code. Click on the hyperlink for the details. Scroll down to find the complete code. The numbers on the left-hand side of the code are the reading frames and the research journal references are hyperlinked as well on the page.
Evaluate the information you have found, considering the accuracy, availability and ease of use.

Cytochrome *b* DNA sequence differences between *Gallotia* lizard populations

	G. stehlini	*G. atlantica*	*G. galloti* (Palma)	*G. galloti* (N Tenerife)	*G. galloti* (S Tenerife)	*G. galloti* (Gomera)
G. atlantica	36	–	–	–	–	–
G. galloti (Palma)	41	25	–	–	–	–
G. galloti (N Tenerife)	40	23	8	–	–	–
G. galloti (S Tenerife)	40	19	10	6	–	–
G. galloti (Gomera)	45	24	19	19	15	–
G. galloti (Hierro)	49	28	19	21	17	4

CHAPTER 09

Continuity of life on Earth

Life first appeared on Earth around 3.5 billion years ago as unicellular organisms. Since then, life has gradually evolved into the vast array of diverse and complex species present today. The genome of a species provides the biological basis for survival and reproduction in the particular environment it occupies. Evolutionary processes therefore involve internal biological factors of species (genetics, structure and function) and external environmental factors that determine the ability of a species to survive in that time and place.

The biological information in the genome that is passed down to offspring, and modified by a range of mutations, provides the variety and adaptability that enables a species as a whole to survive in changing environments. This biological continuity is reflected in the structure and function of species, and can be traced in detail by comparing genomes. By the end of this chapter you will understand how comparative genomics provides evidence of the relatedness of species through evolutionary history.

Syllabus subject matter

WS 4.2.1

Topic 2 • Continuity of life on Earth

EVOLUTION

- define the terms evolution, microevolution and macroevolution
- determine episodes of evolutionary radiation and mass extinctions from an evolutionary timescale of life on Earth (approximately 3.5 billion years)
- interpret data (i.e. degree of DNA similarity) to reveal phylogenetic relationships with an understanding that comparative genomics involves the comparison of genomic features to provide evidence for the theory of evolution.

NATURAL SELECTION AND MICROEVOLUTION

- recognise natural selection occurs when the pressures of environmental selection confer a selective advantage on a specific phenotype to enhance its survival (viability) and reproduction (fecundity)
- identify that the selection of allele frequency in a gene pool can be positive or negative
- interpret data and describe the three main types of phenotypic selection: stabilising, directional and disruptive
- explain microevolutionary change through the main processes of mutation, gene flow and genetic drift.

SPECIATION AND MACROEVOLUTION

- recall that speciation and macroevolutionary changes result from an accumulation of microevolutionary changes over time
- identify that diversification between species can follow one of four patterns: divergent, convergent, parallel and coevolution
- describe the modes of speciation: allopatric, sympatric, parapatric
- understand that the different mechanisms of isolation—geographic (including environmental disasters, habitat fragmentation), reproductive, spatial, and temporal—influence gene flow
- explain how populations with reduced genetic diversity (i.e. those affected by population bottlenecks) face an increased risk of extinction
- interpret gene flow and allele frequency data from different populations in order to determine speciation.

MANDATORY PRACTICAL 4

Analyse genotypic changes for a selective pressure in a gene pool (modelling can be based on laboratory work or computer simulation).

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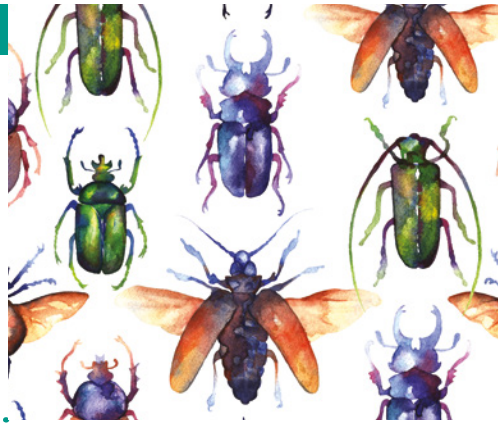
9.1 Evolution

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- define biological evolution, microevolution and macroevolution
- determine episodes of evolutionary radiation and mass extinctions from an evolutionary timescale of life on Earth
- understand the overall process of evolution and that evolutionary change occurs at the level of organisms and genes
- understand that comparison of genomic information provides evidence for the theory of evolution
- interpret data (i.e. degree of DNA similarity) to reveal phylogenetic and evolutionary relationships.



Many life forms have lived and evolved on Earth. The survival of a species is determined by its ability to survive and reproduce in its habitat. **Natural selection** occurs when the pressures of environmental selection confer a selective advantage on a specific phenotype to enhance its survival (viability) and reproduction (fecundity). Over many generations, a species may undergo genetic and phenotypic change. These changes can increase variation and diversity within a species, occasionally causing a new species to arise. Some species persist for long periods. Most species eventually die out and become extinct.

DEFINING EVOLUTION

Charles Darwin (Figure 9.1.1) was an Englishman who sailed as a naturalist on the *HMS Beagle*. He collected specimens while visiting the Galapagos Archipelago, South America, New Zealand and Australia, as well as many other places. Based on his findings during these voyages and the research he performed on domestic pigeons, he developed the theory of **evolution** by natural selection.

Darwin argued that species were not created in their present forms but had evolved from ancestral species. He also proposed a mechanism for evolution, which he termed natural selection, based on two key observations:

1 Members of a population often vary in their inherited traits (Figure 9.1.2).
2 All species produce more offspring than their environment can support, and most of these offspring fail to survive and reproduce (Figure 9.1.3).

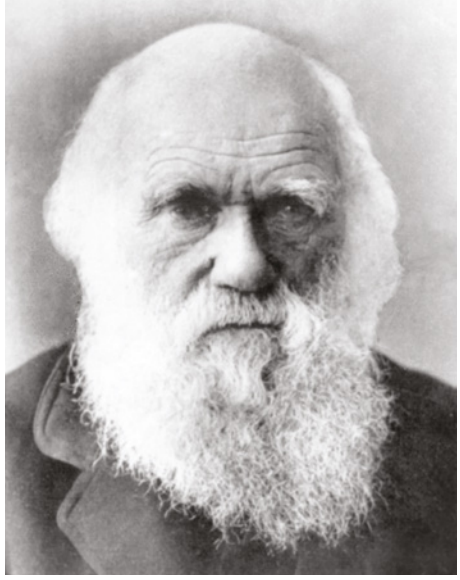
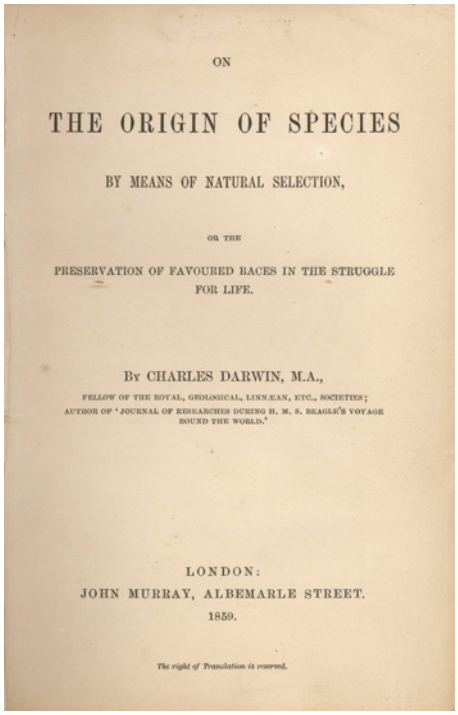


FIGURE 9.1.1 Charles Darwin was a key scientist in the development of the theory of evolution.

FIGURE 9.1.2 Harlequin ladybirds vary in colour and the number of spots.

FIGURE 9.1.3 The green turtle lays between 100 and 200 eggs in a single clutch, and in each season she may lay up to eight clutches. However, not all the eggs will hatch and most hatchlings do not survive to adulthood.

i A naturalist is a person who is an expert or is interested in botany or zoology (the natural world), especially in the field.



ON

THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

PRESERVATION OF FAVOURED RACES IN THE STRUGGLE FOR LIFE.

BY CHARLES DARWIN, M.A.,

FELLOW OF THE ROYAL, GEOLOGICAL, LINNÆAN, ETC., SOCIETIES;
AUTHOR OF 'JOURNAL OF RESEARCHES DURING H. M. S. BEAGLE'S VOYAGE ROUND THE WORLD.'

LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1859.

The right of Translation is reserved.

FIGURE 9.1.4 Charles Darwin published his book *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* in 1859.

A trait is a particular characteristic or feature of an organism.

Evolution is the change in the genetic composition of a population during successive generations, which may result in the development of new species

Based on these two key observations, Darwin drew two inferences:

1 Individuals whose inherited traits give them a higher probability of surviving and reproducing in a given environment tend to leave more offspring than other individuals.
2 This unequal ability of individuals to survive and reproduce will lead to the accumulation of favourable traits in the population over generations.

Collectively, these proposals make up Darwin's theory of evolution by natural selection, also referred to as **Darwinian theory** (Figure 9.1.4). Our modern understanding of evolution was founded on Darwinian theory, but has progressed significantly since then. Biological evolution is now defined as the change in the genetic composition of a population during successive generations, which may result in the development of new species.

Evolutionary change occurs at different levels, referred to as microevolution and macroevolution. In combination, they lead to the genetic variation and diversity required for the appearance of new species.

Microevolution

Microevolution refers to the small-scale variation of allele frequencies within a species or population, in which the descendant is of the same taxonomic group as the ancestor. In other words, microevolution involves genetic and phenotypic changes within a species that do not produce a new species.

This occurs as natural selection and reproduction changes the allele frequency within a population over generations, resulting in species adapted to changing environments. An example of microevolution occurring in Australia today relates to the introduced invasive species, the cane toad (Figure 9.1.5). The majority of the introduced toad population were slow-moving amphibians. However, the toad is evolving longer legs, resulting in an increasing number of longer-legged individuals at the invasion front that are able to travel greater distances more quickly. Mechanisms of microevolutionary change are explored further in Module 9.2.

FIGURE 9.1.5 The cane toad shows evidence of microevolution as subsequent generations of toads in Australia have longer legs than their ancestors.

Macroevolution

Macroevolution is the variation of allele frequencies at or above the species level over geological time, resulting in the divergence of taxonomic groups, in which the descendant is in a different taxonomic group to the ancestor. In other words, macroevolution involves genetic and phenotypic change within species that lead to new species, genera and other taxonomic groups.

Macroevolution is a long-term process by which new species arise. For example, it is supposed all mammals share common ancestry, but have diverged into three major groups: the eutherians (placental mammals), marsupials and monotremes (Figure 9.1.6). Each of these groups comprise many different families, genera and species. Mechanisms of macroevolution are explored further in Module 9.3.

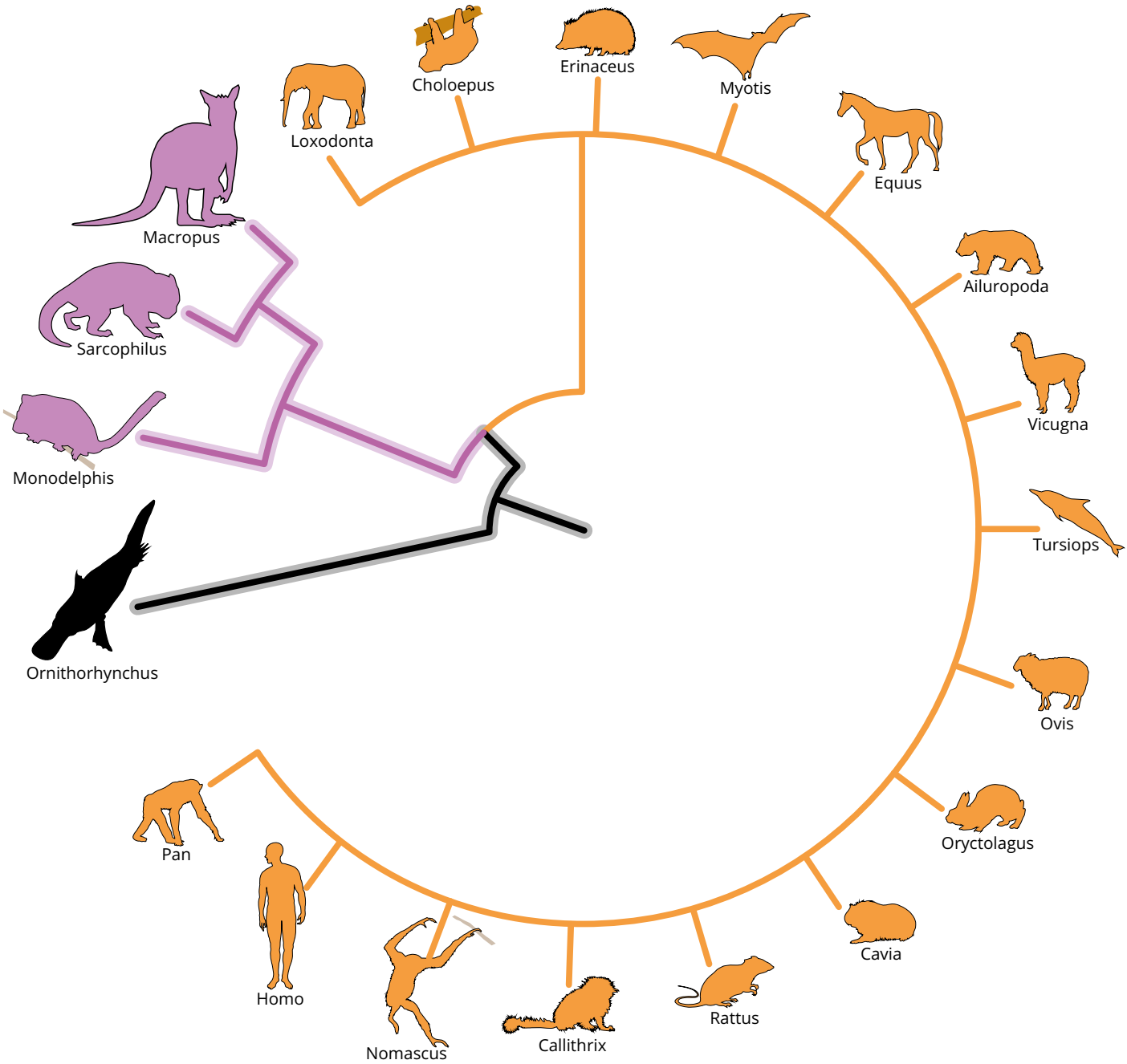


FIGURE 9.1.6 Some genera in the evolutionary tree of mammals. The three main groups are monotremes (black lineage), marsupials (purple lineage), and eutherians (placentals) (orange lineages).

MAJOR EPISODES IN EVOLUTIONARY HISTORY

FIGURE 9.1.7 Ammonite fossils embedded in rock.

The proposed history of Earth and evolving life can be chronologically followed using the **geological timescale**, which covers events that have occurred on Earth from its formation to the present time. The geological timescale is constructed using the order of rocks laid down in a sedimentary rock sequence (a relative timescale in which the oldest rocks are at the bottom) and the fossilised remains of ancient animals and plants within the rock strata. **Fossils** are the preserved remains, impressions or traces of organisms found in rocks, amber (fossilised tree sap), ice or soil (Figure 9.1.7). Petrified wood is fossilised wood.

Geologists use techniques such as radiometric dating to infer an absolute age of rocks, and thus the age of the fossil organisms found within and around them. The age of organic material, such as charcoal and bone, can be estimated by palaeontologists using radiocarbon dating within a limit of 50 000–60 000 years (depending on the instrument and institute).

Geological timescales

The geological timescale is divided into many subdivisions. The largest of these subdivisions is the **eon**. Eons are subdivided into **eras**, which are further subdivided into **periods**, and into still smaller subdivisions called **epochs** (Table 9.1.1).

The subdivisions are often characterised by significant shifts in the predominant taxonomic group or the emergence of new taxa.

The earliest evidence of cellular life, the first bacterial cells, dates to about 3.5 billion years ago in the Archaean eon, including the photosynthetic bacteria that form large colonies that develop into stromatolites, which can still be seen in Western Australia (Figure 9.1.8).

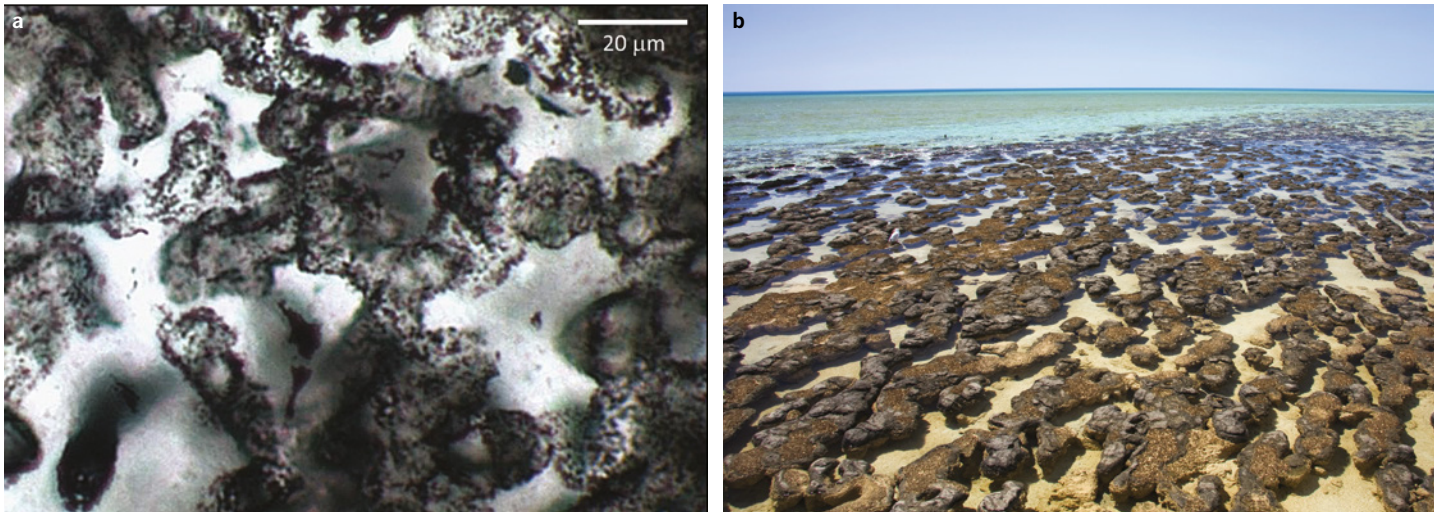


FIGURE 9.1.8 (a) Microfossil of bacteria that lived 3.4 billion years ago found in sandstone at the base of Strelley Pool, Pilbara region, Western Australia. (b) Stromatolites can still be found today at Shark Bay, Western Australia.

Eukaryotic cells first appeared about 2 billion years ago. It is hypothesised that eukaryotic cells evolved from the merging of prokaryotic cells through a process called endosymbiosis. When the prokaryote cells merged, internal membranes formed organelles, such as a nucleus to contain the DNA. Scientists propose that photosynthetic cyanobacteria and aerobic proteobacteria evolved into chloroplasts and mitochondria, respectively, leading to the evolution of more complex multicellular organisms.

Evidence for the first animals appears in the Proterozoic eon. For example, fossils of simple soft-bodied aquatic animals are present in the Ediacaran period. This period is named after the Ediacara Hills region of South Australia, the site of these fossils.

TABLE 9.1.1 Geological timescale in millions of years ago (mya) and the major groups of plant, animal and microbial life for which evidence occurs in the fossil record

Relative duration	Era	Period	Epoch	Age (mya)	Plant life	Animal life
Phanerozoic eon	**Cenozoic**	Quaternary	Holocene	0.01	• modern plants	• evolution of humans
			Pleistocene	2.58		
		Neogene	Pliocene	5.33	• angiosperms dominate forests and grasslands	• mammals diversify, including primates • whales appear in oceans
			Miocene	23.03		
		Palaeogene	Oligocene	33.9	• angiosperms continue to dominate	• many primate groups appear
			Eocene	56.0	• angiosperms continue to dominate	• mammals continue to diversify
			Palaeocene	66.0	• angiosperms continue to dominate	• mammals, birds and pollinating insects diversify
	Mesozoic	Cretaceous		145.0	• angiosperms become dominant	• dinosaurs become extinct • mammals diversify or further develop • birds appear • first primates
		Jurassic		201.3	• conifers abundant • first angiosperms	• age of reptiles, some flying reptiles
		Triassic		252.2	• conifer trees dominate forests	• first mammals • first dinosaurs • reptiles dominate land • amphibians decline
	Palaeozoic	Permian		298.9	• early seed plants develop, including cycads and early conifers	• reptiles diversify • familiar insects develop • many land vertebrates • many invertebrate sea life become extinct
		Carboniferous		358.9	• first large swamp forests of vascular land plants	• insects become more common • first reptiles
		Devonian		419.2	• tree-like vascular land plants, including lycopods • ferns appear	• fishes and coral reefs common
		Silurian		443.8	• first small vascular land plants • many algae	• many coral reefs, shells • first animals on the land—amphibians and invertebrates
		Ordovician		485.4	• types of large algae found as fossils	• many invertebrates • first vertebrates—fishes—found
		Cambrian		541.0	• more types of algae appear	• animals with bodies protected by shells • first fishes appear
Proterozoic eon		Ediacaran		635	• some algae	• soft-bodied animals • a few fossils found of animals with jelly-like bodies
				2500	• first animal traces • multicellular life develops in shallow warm seas • fossils rarely found, due to the age of the rocks and the soft fragile bodies of these organisms	
Archaean eon				4000	• bacteria (prokaryotes) abundant • fossilised and living stromatolites are still found on Earth today • oldest known sedimentary rocks, and oldest 'fossil' remains—chemical traces of living things	
Hadean time				4600	• solidification of the Earth from a ball of molten rock	

Note: The Proterozoic eon, Archaean eon and Hadean time are collectively known as Precambrian time. Hadean time is not a geological era, eon or period.

Vascular plants appear later, in the Paleozoic era. These were seed plants, such as cycads and conifers. Evidence for flowering plants appears more recently in the Mesozoic era (Figure 9.1.9).

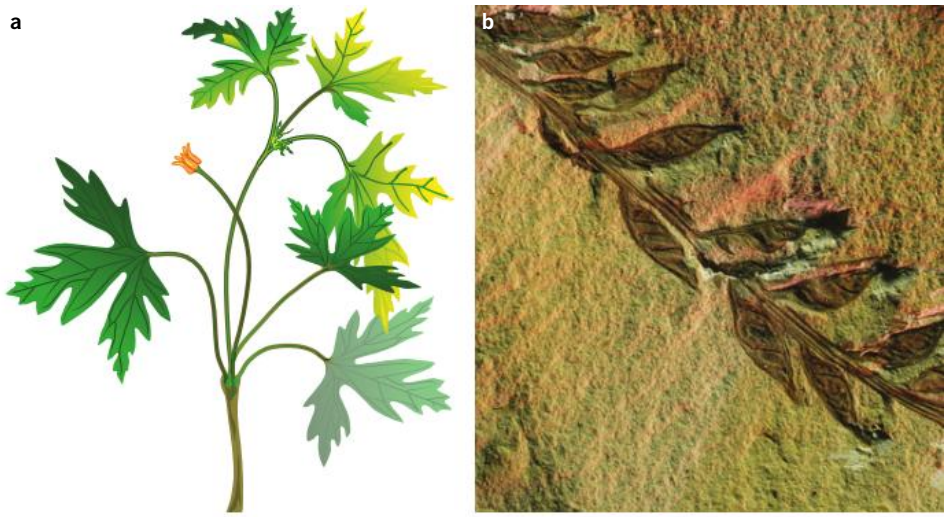


FIGURE 9.1.9 Earliest flowering plants from the Cretaceous period: (a) *Leefructus mirus* and (b) fossil of *Archaefructus* sp.

A lineage is all the species that are descendants of a common ancestor.

While mammals first appear in the **fossil record** in the Triassic period, along with the dinosaurs, the human ancestors appear and diversify in relatively recent time. Hominins (the human lineage) arise in the Neogene period and modern humans are first evident around 200 000 years ago during the Holocene epoch of the Quaternary period (the period we are in now).

EVOLUTIONARY RADIATION

An evolutionary radiation is an increase in taxonomic diversity and morphological variation, such as new body types and metabolisms. It may be slow or rapid, and can affect one clade or many.

Throughout evolutionary time, new species with genetic and phenotypic variations arise. The evolution of a new species is known as **speciation**. Genetic diversity provides the basis for species to thrive in new habitats.

Evolutionary radiation is the increase in new morphological types or taxonomic diversity over time. It may be slow or rapid (in geological time), and may occur when a new ecological space becomes available to a population or when populations adapt.

The fossil record indicates several evolutionary radiations (Table 9.1.2). Evolutionary radiations may occur over long periods or they may occur in more rapid bursts (on a geological timescale), such as the emergence of 36 lineages of modern birds in 10–15 million years in the early Palaeogene period.

Many of these radiations, and other smaller recognised radiations, coincide with geographical, climatic and biotic changes such as:

- continental movements that create whole new aquatic, coastal and terrestrial ecosystems
- dramatic loss of species, such as a mass extinction (see page 441)
- major climatic change, such as periods of global cooling and global warming
- atmospheric change, such as fluctuations in carbon dioxide and oxygen concentrations.

One example is the radiation of modern birds (the Neonaves group that includes songbirds and parrots, but not the ratites or flightless birds) after the mass extinction at the end of the Cretaceous 66 mya, indicated by both the fossil record and recent genome sequencing of living birds (Table 9.1.2).

TABLE 9.1.2 The major evolutionary radiations

Evolutionary radiation event	Time	Taxa
Ediacaran biota expansion	• 575 mya • pre-Cambrian era, after 'snowball earth'	• earliest multicellular communities; early aquatic animals • appearance of mobility, multicellular heterotrophs, start of skeleton formation, sexual reproduction, complex ecosystems
Cambrian explosion	• 530–520 mya	• divergence of most metazoan phyla (metazoan are animals with discrete developmental stages)
Great Ordovician Biodiversification Event (GOBE)	• ~500 mya	• radiation of animal life • Cambrian fauna declined and were replaced with a fauna rich in filter feeders such as krill and open ocean fish
Mesozoic–Cenozoic Radiation	• 66 mya	• radiation of land plants after their colonisation of land, mammals and some reptiles
Cretaceous angiosperm radiation	• 125–65 mya	• radiation of angiosperms • further diversification of insects
radiation of placental mammals **radiation of modern birds**	• starts 66 mya • end of Cretaceous after dinosaur extinction	• mammal radiation from small shrew-like mammals to diversity including horses, bats and whales by the Eocene epoch • rapid radiation of modern birds; 36 lineages in 10–15 mya

Radiations may occur when new ecological space becomes available as a result of the activity of other species. For example, the fossil record shows a group of bivalves (the class that includes clams and oysters) that first appeared in the Silurian (around 440 mya) started to diversify much later, in the Cretaceous (~145 mya). This coincided with the growth of seagrasses and mangroves, which changed the seabed habitat to favour diversification of the bivalves.

The initiation of new morphology and biochemistry through genetic change is proposed to provide the capacity to radiate (Figure 9.1.10). This may include new developmental patterns or new body plans, such as legs rather than antennae, or six legs rather than eight legs. Mutations in gene families controlling developmental patterns, such as the *Hox* genes (Chapter 5), may be important in the evolution of new body plans. Smaller-scale genetic changes, such as a new enzyme providing the ability to metabolise a new food source, can allow a species to inhabit a new ecological space.

i Evolutionary timescales are measured in 'million years ago' (mya).

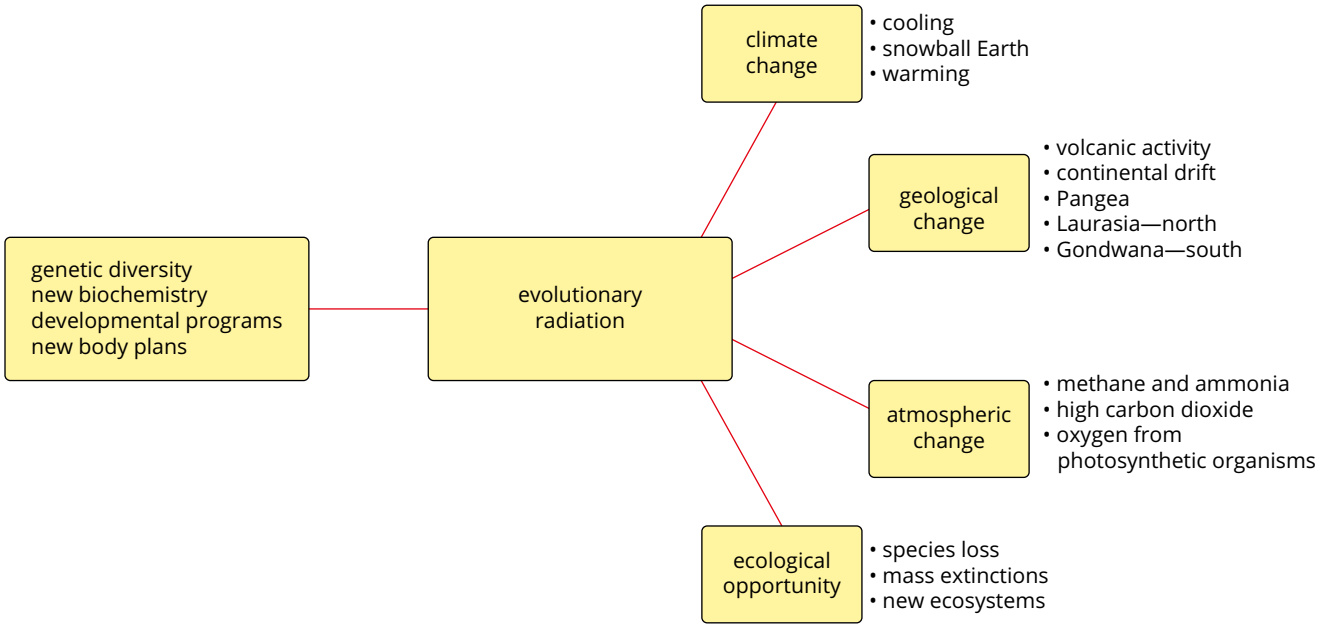


FIGURE 9.1.10 Genetic diversity leads to biological diversity, which provides the capacity to radiate into ecological spaces that become available through a range of geological, climatic and biotic factors.

The fossil record provides evidence for evolutionary radiation of mammals after the dinosaurs went extinct at the end of the Cretaceous period (Figure 9.1.11). The first small mammal diversification generated seven new taxa, which are proposed to have radiated into new environments before the end of the Cretaceous period (66 mya). The Condylarthra clade, for example, underwent evolutionary radiation soon after, between 45 and 65 mya, resulting in 12 branches of taxa.

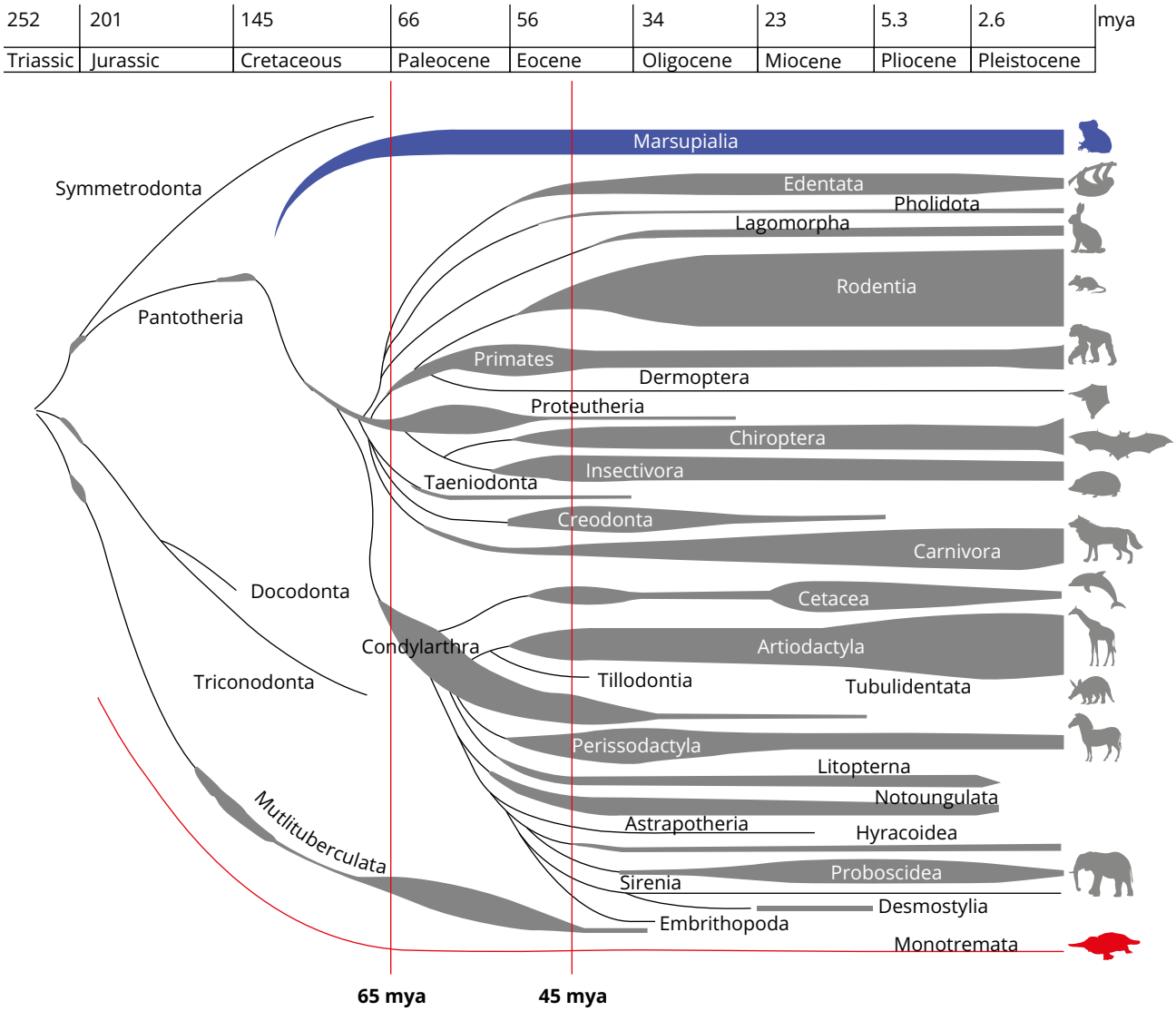


FIGURE 9.1.11 A diagram representing the evolutionary radiation of mammals through the Cenozoic era. The width of the bar indicates the number of species in each group at different time periods. The timescale is in mya (million years ago).

WS 4.2.2

EXTINCTION

When the phenotype, viability or reproductive success (fecundity) of a species fails to change in correlation with environmental changes, a species can die out. Loss of all populations of a species or groups of species is called **extinction**. Extinction can occur as a result of changes in the physical environment or from changes in ecological interactions between species, such as the arrival of a new predator or competitor. The average life of a species varies depending on the type of organism, but is generally a few million years according to interpretations of fossil records.

Some genera have persisted over much longer periods. For example, the coelacanth group dates back to the Devonian period of about 400 mya (Figure 9.1.12). They have shown continued reproductive success in their habitats in deep-water caves, some of which remained stable during ecological events that caused extinction of other species. The average rate of natural loss of species is called the **background extinction**. The background species extinction rate, based on data from the fossil record, is 0.1–1 species extinction per million species per year, or 0.1–1 E/MSY.

FIGURE 9.1.12 The coelacanth inhabits steep rocky shores in the western Pacific and Indian oceans, living at depths of 150–700 m. An adult coelacanth may reach a length of 2 m.

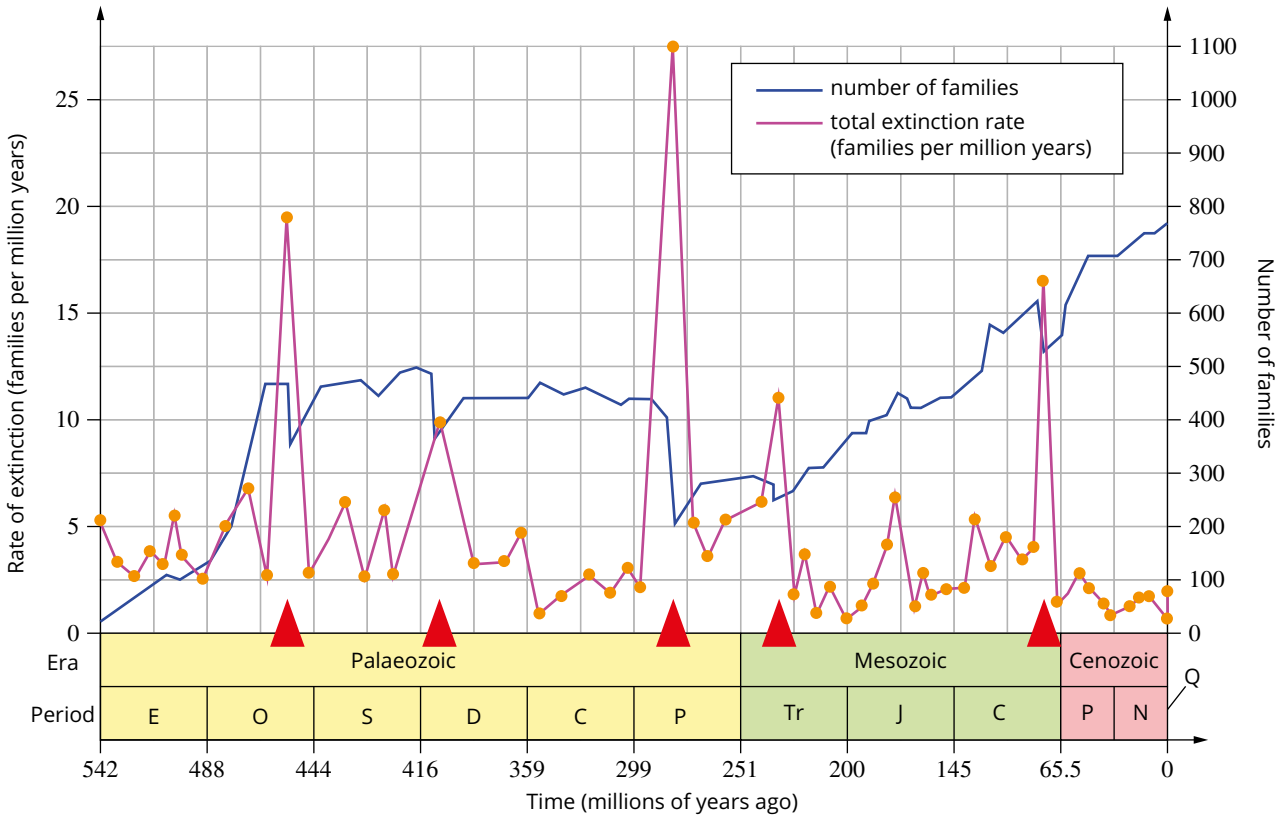
Mass extinctions

Throughout the fossil record, there is evidence of significant mass extinction events. **Mass extinctions** are episodes of large-scale extinctions where loss of taxa exceeds the background extinction rate. They usually follow disruptive changes to the global climate, loss of sea or land due to the shifting of continents (plate tectonics) or other major impacts on ecosystems.

Mass extinctions, like the one that wiped out most of the dinosaurs at the end of the Cretaceous period, led to 'empty' ecological niches. The sudden availability of resources and reduced competition or predation may have been advantageous for some of the remaining species. Mass extinctions can also result in surviving populations being small, and thus genetic drift (random changes in allele frequency) in founding populations can lead to rapid evolution (Module 9.2). Periods of significant evolutionary radiation often follow mass extinction events. For example, the period after the Triassic extinction about 230 mya shows a marked increase in the number of families in the fossil record.

Five mass extinction events are evident from the fossil record, and in each more than 50% of hard-bodied marine species became extinct (Figure 9.1.13). During the Permian period (299–252 mya), it is thought all of the continents came together and shallow continental seas were gradually lost. This large land mass (called Pangaea) has been proposed to have caused reduced rainfall, temperature extremes, harsh conditions and the death of many species including the extinction of marine trilobites. The fossil evidence for trilobites is particularly well-documented, as hard-bodied organisms fossilise well.

FIGURE 9.1.13 The five generally recognised mass extinction events, indicated by red arrowheads, represent peaks in the extinction rate of marine animal families (purple line and left vertical axis). These mass extinctions interrupted the overall increase in the number of marine animal families over time (blue line and right vertical axis).



Recent extinctions

In recent times, humans have also been the cause of species extinctions at an accelerating rate. Since European settlement in Australia, dozens of Australian mammal species have been lost through a combination of factors, including the introduction of cats, foxes and the cane toad. Plants, birds and other animal species have also become extinct as a result of land clearing, habitat loss and altered fire regimes.

Species conservation status

The conservation status of a species is used to indicate how at risk a species is of becoming extinct, or whether a species is extinct (Table 9.1.3). When a species population is too low to be maintained in the wild, the species is considered endangered. Species may become extinct in the wild but survive in captivity, such as in zoos and captive breeding programs. These species are considered to be extinct in the wild. Scientists and conservationists use these categories to monitor the status of species and biodiversity in ecosystems, particularly in relation to the role of human activity in a species vulnerable to extinction.

TABLE 9.1.3 Categories of species conservation status based on IUCN red list categories and criteria*

Abbreviation	Conservation status	Description of status
EX	extinct	• no known surviving individuals
EW	extinct in the wild	• known individuals only known to survive in captivity, or as a naturalised population outside its historic range
CR	critically endangered	• extremely high risk of extinction in the wild • reduced population size, very low number of mature individuals, fragmented geographical range, predicted within 10 years
EN	endangered	• high risk of extinction in the wild
VU	vulnerable	• high risk of becoming endangered in the wild
NT	near threatened	• likely to become endangered in the near future
LC	least concern	• lowest risk group • widespread and abundant taxa are included in this category
DD	data deficient	• insufficient data to assess the risk of extinction
NE	not evaluated	• not yet evaluated against the criteria

* The Red List of Threatened Species is developed by the International Union for Conservation of Nature (IUCN).

CASE STUDY 9.1.1

Are humans causing the Sixth Mass Extinction?

The decline in biodiversity has reached critical levels, with evidence of a marked increase in species extinctions in modern times. Researchers are investigating whether humans are now the main cause of recent species extinctions. Making an objective assessment of human impact on species extinction relies on knowing the rate of background extinction before humans appeared.

A research study published in 2015 addressed two main questions:

1. Are the modern rates of mammal and other vertebrate extinctions higher than the background rate?
2. Have the extinction rates of mammal and other vertebrate groups changed through time?

The researchers analysed prehistoric extinction rates, reassessed and revised the background extinction rate, and analysed current data on biodiversity from the International Union for Conservation of Nature (IUCN). IUCN, an international organisation that monitors the health status of environments and species, publishes the Red List of species that are endangered, extinct in the wild and extinct. The researchers analysed historical records and data up to 2014, so when their report says 'since 1500' it means for the time period 1500–2014.

Background extinction rates

The researchers calculated the background extinction rate at two species extinctions per million species per year. This is double the value previously used and is therefore less likely to overestimate the rates of modern extinction over the background extinction rate.

Modern extinction rates

The researchers separately analysed data for species recorded as extinct (the most conservative estimates of human impacts) as well as data including species that are extinct in the wild and critically endangered. Here we will mainly focus on the data for extinctions only (not the data for nearly extinct and extinct in the wild).

The number of species extinctions recorded 'since 1500' (that is, from 1500 to 2014, a period of 514 years) are listed in Table 9.1.4. The researchers subdivided the data to record the number of these extinctions that had occurred just since 1900 (that is, from 1900 to 2014, a period of 114 years).

TABLE 9.1.4 Number of extinctions in different taxa in the period 1500–2014. 'Since 1500' means 1500–2014. 'Since 1900' means 1900–2014. Data is from the IUCN Red List 2014.

Vertebrate taxon	Number of extinctions	
	Since 1500	Since 1900
amphibians	34	32
birds	140	57
fishes	66	66
mammals	77	35
reptiles	21	8

The researchers then calculated the rate of extinction for each of the animal taxa compared to the background extinction rate (Table 9.1.5). If the extinction rate for the time period is the same as the background rate, the value will be zero (no increase in extinction rate). They graphed the data of cumulative extinctions at different time intervals (Figure 9.1.14 on page 444).

TABLE 9.1.5 Increased rate of extinction above background extinction rate since 1500 and 1900. A value of zero means no increase in extinction rate above background rates. Data is from the IUCN Red List 2014.

Vertebrate taxon	Evaluation of modern extinction rates above background	
	Since 1500	Since 1900
amphibians	5	22
birds	13	24
fishes	5	23
mammals	14	28
reptiles	5	8

continued over page

CASE STUDY 9.1.1

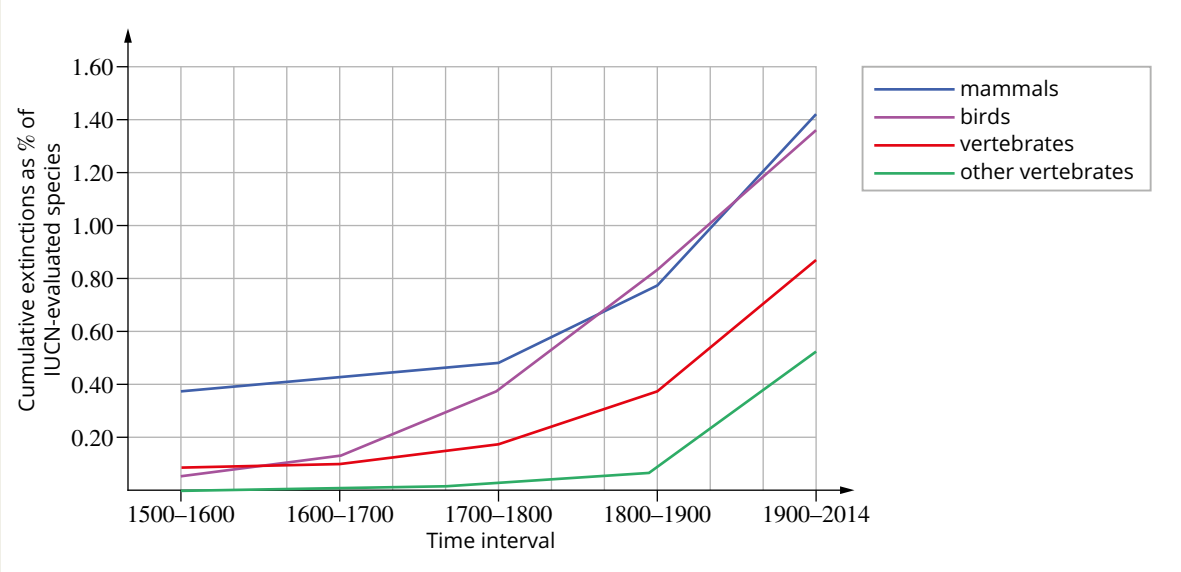


FIGURE 9.1.14 Increased rate of extinctions above background extinction rate for different taxa.

The researchers then asked a third question. How long would it have taken for modern extinctions (those that occurred in the last 114 years) to have occurred if the extinction rate had not increased above the background extinction rate? The calculations from their data indicate that, if the extinction rates had not increased since 1900, it would have taken hundreds or thousands of years for these vertebrates to have become extinct.

Review

1. View the data for 1900–2014 in Table 9.1.4 (page 443). Organise the vertebrate taxa in order of least extinctions to most extinctions.
2. Calculate the proportion of extinctions that have occurred since 1900 for each group of all extinctions since 1500. Copy and complete the table to record your results. Record your results to the nearest whole percentage.

Extinctions since 1900, by taxon

Taxon	Proportion (%) extinct since 1900
mammals	
birds	
reptiles	
amphibians	
fishes	

3. Interpret the data and state your conclusion concerning the first question asked by the researchers.
4. Analyse the data in Figure 9.1.14. Determine the time period in which the rate of extinction is greatest.
5. Based on the data presented, draw a conclusion to the second question asked by the researchers. Use the data to explain your conclusion.

EVOLUTIONARY RELATEDNESS AND PHYLOGENY

As you learned in Chapter 2, homologous features provide clues to common origins or shared ancestry and are used to construct phylogenetic trees to depict the evolutionary relationships. Comparative morphology involves comparing the structures of living organisms and fossil evidence of extinct species to infer evolutionary relationships. Comparative genomics involves the comparison of DNA sequences, to infer evolutionary relationships and processes.

Comparative genomics

Molecular homology is the similarity in the DNA and proteins of organisms. Today it is possible to sequence DNA and compare individual genes or whole genomes. Comparing genomic features to determine evolutionary relatedness is called **comparative genomics** (Figure 9.1.15). Genomic features include whole genomes, individual gene sequences, regulatory sequences, the presence of gene duplications and the presence or absence of non-coding DNA. Non-coding DNA includes introns, transposable elements (which are DNA sequences that move between genomes and can be permanently inserted into the genome of a lineage) and repetitive DNA sequences such as short tandem repeats. Genome-wide association studies scan entire genomes to detect single base changes, or **single nucleotide polymorphisms (SNPs)**, which produce new alleles.

If species have a very similar set of proteins, chromosomes or DNA sequences it is interpreted as evidence that they shared a recent **common ancestor**. 'Recent', in evolutionary terms, may be hundreds of thousands, even millions, of years. You will encounter these comparisons in the evidence of genetic relatedness, which is used to construct phylogenetic trees and infer patterns of evolution.

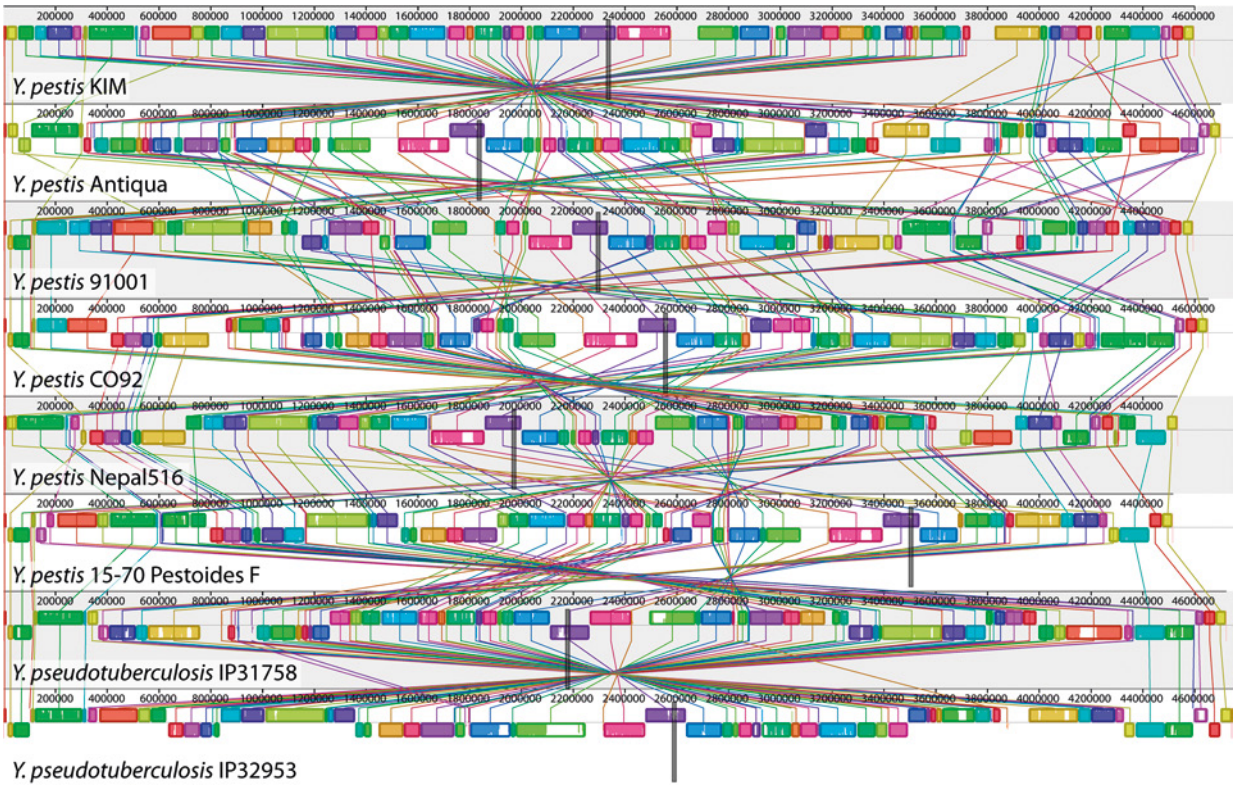
Homologous features, which can be physical structures or molecules such as protein and DNA, are similar because the organisms share a common ancestor. Homologous features are retained through divergent evolution (divergence from a common ancestor).

Taxonomy and systematics are the branches of science involved in classifying organisms and constructing evolutionary trees. They now use both morphological and molecular data to identify common ancestry and evolutionary relationships.

Molecular homology is the similarities in the sequences of DNA and protein molecules between organisms.

Comparative genomics is the field of biological research that uses information from the genomes of organisms to study biological similarities and evolutionary relationships.

FIGURE 9.1.15 Comparative genomics with whole genome alignment of *Yersinia* bacteria. *Yersinia pestis* is the bacterium that causes plague disease, which has spread in several epidemics throughout history and still appears in many countries. This alignment compares the genomes of several related bacteria.



Constructing phylogenetic trees from genomic data involves lining up the sequences from different species next to each other to identify the similarity and difference in base sequence. This is followed by computational statistical analyses to calculate and compare features such as the percentage difference between species, the degree of divergence or how much the DNA has changed since species formed a new lineage from the common ancestor.

A simplified example to show the procedure for constructing a phylogenetic tree from a multiple sequence alignment is shown below. The gene sequence is for casein, a milk protein, from three artiodactyls (hoofed mammals): a camel, a pig and a peccary. The steps describe the sections in Figure 9.1.16.

a A short segment of the gene from each organism is sequenced, the data is entered into a database and a computer program aligns the sequences. The conserved bases (those that are the same in all sequences being compared) are marked with an asterisk. These can be ignored when counting base differences.

b The number of base differences is recorded in a data matrix. Researchers perform computational analysis on the number of differences (or other features of the DNA being recorded) before preparing the tree; for example, calculating percentage of base changes to compare across different data sets and accounting for gaps in different sequences.

c A rooted tree indicates an estimated distance from the common ancestors of each pair of organisms. One possible way to draw this tree is shown in (c). The scale on the horizontal branches is the number of base changes, for example the 6-base difference between pig and peccary appear as the 2 and 4 units of distance on the horizontal branches from the closest node. This represents the distance from the recent common ancestor of these two species. Likewise, the 13-base differences between pig and camel are 2 + 3 + 8 on the horizontal bars. Try yourself to find the 15-base differences between peccary and camel.

d Unrooted trees show the genetic distance between each organism in the analysis. An unrooted tree does not show a common ancestor. The number of base differences is shown by the length of each branch from the node.

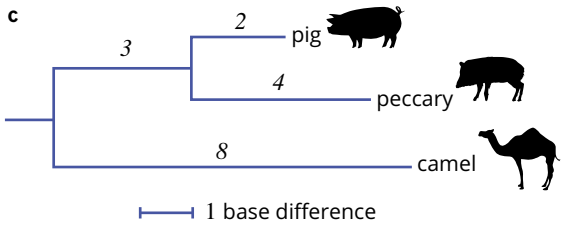
These trees indicate that pigs and peccaries are more closely related to each other than either one is to the camel.

a

```
Camel   TGTCCCCAAAACTAAGGAGA   CCATCATTCCTAAGCGCAAA   GAAATGCCCTTGCTTCAGTC
Peccary AGACCCCAAACCTAAGGAGA   CCGTTGTTCACAAGCGTAAA   GGAATGTCCTCCCCTAAATC
Pig     AGATTCCAAAGCTAAGGAGA   CCATTGTTCCCAAGCGTAAA   GGAATGCCCTTCCCTAAATC
         *    *****  *********  **  *   ***   *****  ***  *  ****  ***   *  *  *  **
```

b

	camel	peccary	pig
camel	–	15	13
peccary		–	6
pig			–



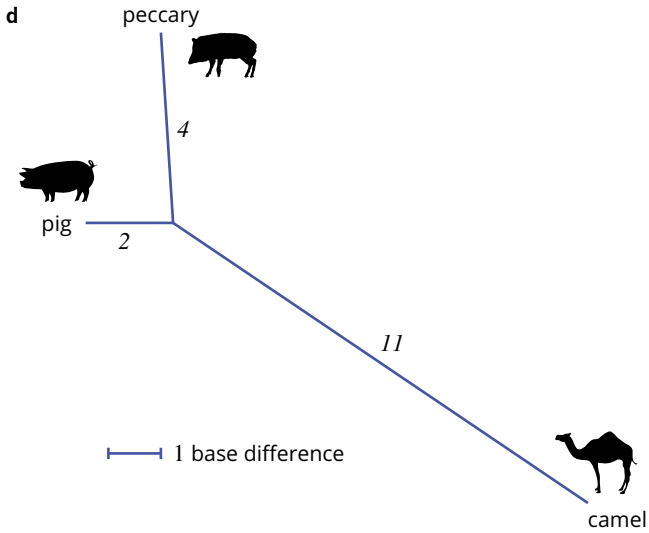


FIGURE 9.1.16 Constructing a phylogenetic tree from DNA sequences. (a) Multiple sequence alignment. (b) Data matrix of base differences between each pair of sequences. (c) A rooted tree with a scale bar. (d) An unrooted tree with a scale bar. The number of base differences on each arm of the tree is indicated in (c) and (d).

Molecular phylogenetics has some limitations:

- Nucleotide changes may reverse, so that two sequences can appear identical, as though no changes have occurred, but in fact two base changes have occurred. This underestimates the evolutionary distance between the species.
- The very large quantities of data obtained from DNA and whole genome sequencing requires rigorous data collection and storage, accurate matrix organisation, specialised software and large computational power to generate phylogenetic trees.

The following section describes some examples of using a comparative genomic approach to construct and/or clarify phylogenetic trees.

Using molecular data to resolve polytomies

In Chapter 2 you saw an example of a polytomy in the ray-finned fishes. This is where multiple branches come from a node because there is not enough information to distinguish them.

Recent phylogenetic analysis of these fish using genomic data helps clarify the tree. Researchers obtained molecular and genomics data from nearly 2000 fish (imagine the data matrices needed) to construct a new phylogeny, part of which is shown in Figure 9.1.17. Compared to the tree shown in Figure 2.2.25 (page 42), you will notice that several taxa names are changed with the new analysis (two species are highlighted for comparison between the trees). The new molecular data provides more evidence to infer distinct lineages or clades within the polytomy. This section of the tree no longer looks like the garden rake.

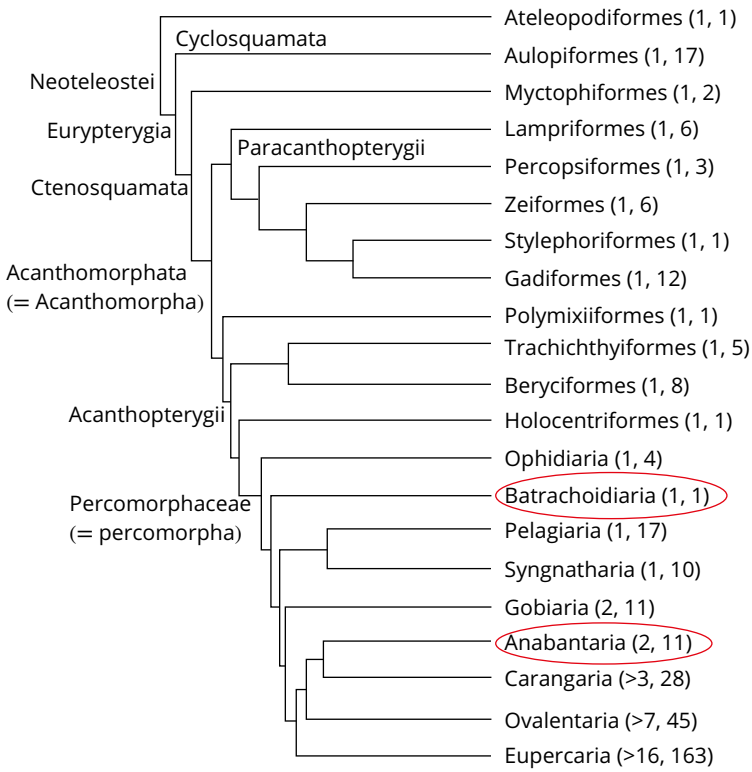


FIGURE 9.1.17 Part of the new phylogeny for ray-finned fishes based on genomic data (numbers in parentheses indicate number of orders and families included in each major clade, respectively). Some previous polytomies are now organised into separate groups. For example, the two circled groups, toadfish (Anabantaria) and bettas (Batrachoidiaria), are now separate groups. Taxon names may differ from the previous tree.

WS 4.2.3

CASE STUDY 9.1.2

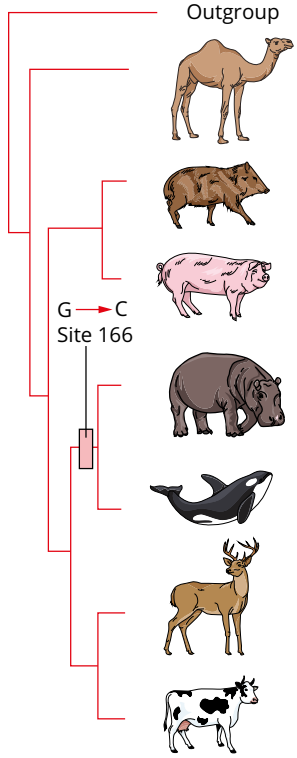
The case of the whale and the hippo

A multiple sequence alignment of the casein milk protein gene in seven species is illustrated in Figure 9.1.18a. The data matrix in Figure 9.1.18b shows that, in this small portion of the gene, there are three base differences between the whale and hippopotamus and 11 base changes between the whale and the camel. The result of this gene analysis (and several other types of DNA) confirms that the whale is most closely related to the hippopotamus and therefore is within the artiodactyl (even-toed hooved mammals) order, not a sister group. A single base change shared by the whale and hippopotamus place them together in a clade.

Review

1. Explain how the casein gene sequence refutes the proposal that the whale is more closely related to the camel than any other hooved animal (artiodactyls), a conclusion which was based on phylogeny construction from ankle bone structure.
2. Describe some of the limitations of the molecular analysis shown in this example.
3. Suggest additional information that would make the molecular analysis stronger.

a



	142	162 166 177	192
Outgroup	AGTCCTCCAAACTAAGGAGA	CCATCTTTCCTAAGCTCAAA	GTTATGCCCTCCCTTAAATC
Camel	TGTCCCCAAAACTAAGGAGA	CCATCATTCCTAAGCGCAAA	GAAATGCCCTTGCTTCAGTC
Peccary	AGACCCCAAACCTAAGGAGA	CCGTTGTTCACAAGCGTAAA	GGAATGTCCTCCCCTAAATC
Pig	AGATTCCAAAGCTAAGGAGA	CCATTGTTCCCAAGCGTAAA	GGAATGCCCTTCCCTAAATC
Hippo	AGTCCCCAAAGCAAAGGAGA	CTATCCTTCCTAAGCATAAA	GAAATGCCCTTCTCTAAATC
Whale	AGTCCCCAXAGCTAAGGAGA	CTATCCTTCCTAAGCATAAA	GAAATGCGCTTCCCTAAATC
Deer	AGTCTCCGAAGTGXAGGAGA	CTATGGTTCCTAAGCACGAA	GAAATGCCCTTCCCTAAATA
Cow	AGTCCCCAAAGTGAAGGAGA	CTATGGTTCCTAAGCACAAG	GAAATGCCCTTCCCTAAATA

b

	camel	peccary	pig	hippo	whale	deer	cow
camel	–						
peccary	15	–					
pig	13	6	–				
hippo	12	13	10	–			
whale	11	12	11	3	–		
deer	18	18	14	10	11	–	
cow	14	15	13	7	9	5	–

FIGURE 9.1.18 (a) A section of the sequence alignment. Blue letters indicate conserved (unchanged) bases across all the species. Red letters show conserved bases that help identify clades. X in the genetic code represents an unknown base. The single base change at position 166 identifies the whale and hippopotamus as a clade. The phylogenetic tree constructed from the results is shown on the left. (b) Data matrix showing number of base changes between paired sequences determined from the multiple sequence alignment of a milk protein gene in the whale and six artiodactyl species.

CASE STUDY 9.1.3

The radiation of the flightless birds

Ratites are flightless birds living in several southern hemisphere countries. They include the ostrich in Africa, the emu and cassowary in Australia, the rhea in South America and the kiwi in New Zealand. Despite an extensive ratite fossil record and many theories about their evolutionary radiation, questions remain about the evolutionary relatedness of living and extinct species. Scientists have been unable to extract DNA from the fossil skeletons of extinct ratites. However, they have recently isolated and sequenced mitochondrial and nuclear DNA from fossilised egg shells of the extinct elephant bird (*Aepyornis*) from Madagascar, an island off the east coast of Africa. They can now determine the closest living relative of the elephant bird (Figure 9.1.19).

FIGURE 9.1.19 Illustration of the extinct ratite, the elephant bird from Madagascar, based on fossil evidence and relationship to extant ratites.

A 40-base DNA sequence from three extant ratites (rhea, kiwi and cassowary) and the extinct elephant bird is shown below in a multiple sequence alignment.

```
Rhea          AATAGTT-CATAAATATCCGGCACCATAAATCAGCGCCTA
Kiwi          AATAGTC-CGTAAATATCTGGTTCCATAAATTAACGCAAA
Cassowary     AATAGTT-CATAAATATCCGACCCCATAAATTAACGCGAA
Elephant bird AATAGTG-TTTAAATATCTGGAACCCAGACCACATACAAA
```

The researchers constructed a phylogenetic tree based on all of the mitochondrial and nuclear sequence data available from all extant and extinct ratites for which DNA is available. A simplified phylogenetic tree of the major taxa is shown in Figure 9.1.20.

Review

1 Copy the data matrix table below into your workbook. Count the number of base differences for each pair of birds and record the numbers in the data matrix.

	rhea	kiwi	cassowary	elephant bird
rhea	–			
kiwi		–		
cassowary			–	
elephant bird				–

2 Based on your counts of this sequence, identify and justify the living species that is the closest relative of the extinct elephant bird.

3 Explain whether your determination of the closest relative of the elephant bird from the sequence alignment agrees with the phylogenetic tree in Figure 9.1.20.

4 Compare the result for the cassowary and rhea obtained from the short DNA sequence and the phylogeny produced from the full sequence data. Identify any discrepancy or disagreement between these results and suggest a reason for any discrepancy.

5 State the time in mya and identify the geological period of the common ancestor of the elephant bird and kiwi from Figure 9.1.20.

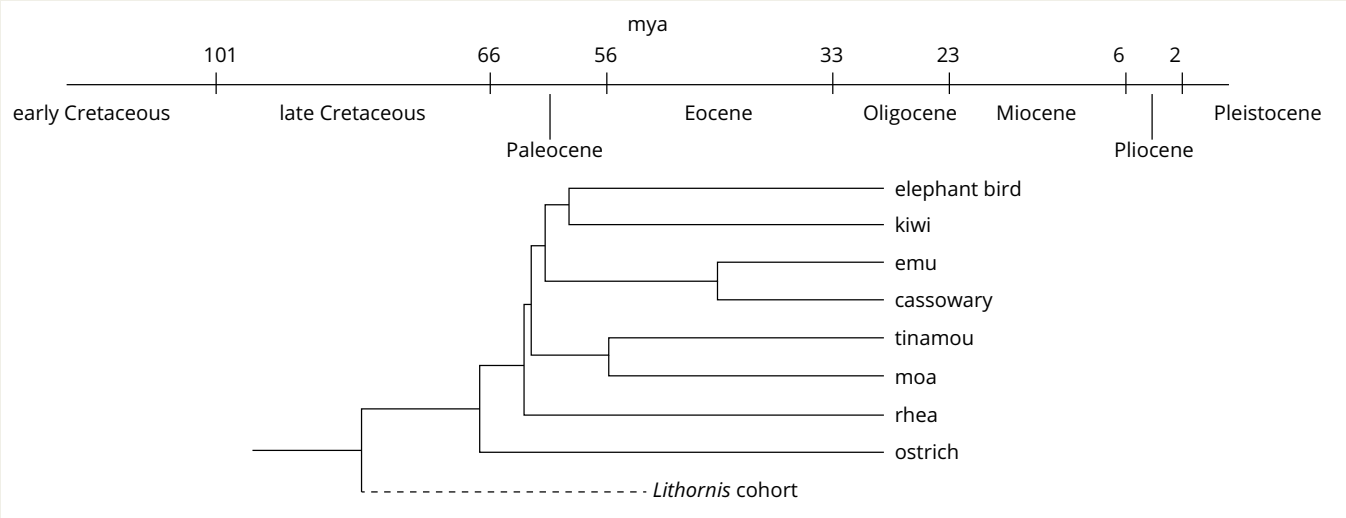


FIGURE 9.1.20 Phylogenetic tree of representative ratites based on genomic data.

9.1 Review

SUMMARY

- Biological evolution is the change in the genetic composition of a population during successive generations, which may result in the development of new species.
- Microevolution is the small-scale variation of allele frequencies within a species or population.
- Macroevolution is the variation of allele frequencies at or above the species level over geological time, resulting in the divergence of taxonomic groups.
- The history of life on Earth follows the geological timescale, which is subdivided into eons, eras, periods and epochs.
- Evidence for the history of life on Earth comes from the fossil record. Organisms appear sequentially in the fossil record over time in increasing complexity.
- Background extinction is the average rate of natural loss of species. Extinction can occur as a result of changes in the physical environment or in the interaction between species.
- Mass extinctions are large-scale extinctions following disruptive changes to global climates and land masses. Five mass extinctions are recorded in the fossil record.
- Evidence indicates human activity is responsible for loss of biodiversity and the sixth mass extinction.
- Morphology of fossilised, extinct and living organisms provides evidence of evolutionary relatedness, as do similarities in DNA and protein sequences between organisms.
- Evolutionary radiation is an increase in taxonomic and morphological diversity over geological time. It may be fast or slow (in geological time).
- Phylogenetic trees show the evolutionary relationship between different groups of organisms based on morphological and molecular homology. The branches and nodes of phylogenetic trees indicate common ancestry between organisms.

KEY QUESTIONS

Retrieval

1 Define evolution.

2 Define microevolution.

3 Identify the eon in which multicellular animals and plants first appeared.

4 Define mass extinction.

5 Define evolutionary radiation.

6 Recall when eukaryotic cells first appeared.

7 Identify the era and time of the radiation of animals with discrete developmental stages (metazoan) from Table 9.1.2 (page 439).

8 List the types of DNA that can be used for comparative genomics.

Comprehension

9 Describe the difference between microevolution and macroevolution.

10 Explain why identifying more than 5000 species of songbirds illustrates macroevolution rather than microevolution.

11 Explain how comparative genomics is used as evidence for the theory of evolution.

12 Summarise the environmental factors that influence the progress and direction of evolutionary radiations.

13 Describe comparative genomics.

Analysis

14 Interpret the geological timescale and species chart in Table 9.1.1 on page 437 to identify the period in which the earliest land (terrestrial) plants appeared.

15 Use Table 9.1.2 (page 439) to identify the significant pre-Cambrian Ediacaran biota expansion in relation to evolutionary radiation.

16 **a** Use the information in Figure 9.1.13 (page 441) to describe what happened to marine animals during the first half of the Oligocene period (488–460 mya).

b Identify this evolutionary process.

c Explain the changes in marine animal diversity just after this, at around 450 mya.

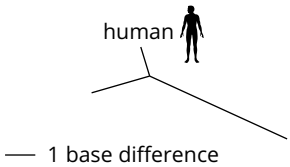
17 Compare the three sequences below.

```
monkey   ATGCACACCTCCATTATA
gorilla  ATGTACGCTACCATAACC
human    ATGCACACTACTATAACC
         *** ** *  * ** *
```

a Complete the data matrix of number of base changes.

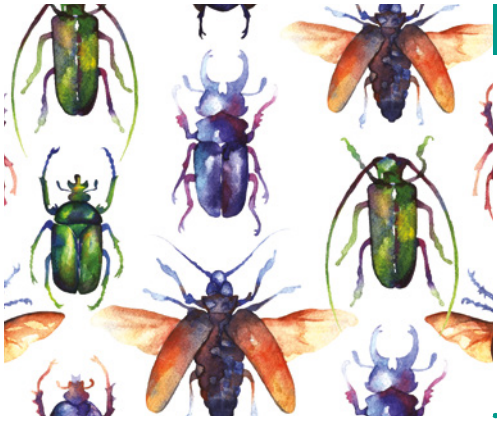
	monkey	gorilla	human
monkey	–		
gorilla		–	
human			–

b Complete the unrooted phylogenetic tree for these three species based on the data provided.



c Determine which species is more closely related to the gorilla from the provided data.

9.2 Natural selection and microevolution



BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recognise that natural selection occurs when environmental selection pressures confer a selective advantage on a specific phenotype to enhance its survival (viability) and reproduction (fecundity)
- identify that a selection of allele frequency in a gene pool can be positive or negative
- interpret data and describe the three main types of phenotypic selection: stabilising, directional and disruptive
- explain microevolutionary change through the main processes of mutation, gene flow and genetic drift.

To some extent, all physical characteristics of an organism are controlled by their genetic make-up. Individuals of the same species tend to have strong similarities in their genetic make-up, but are not identical. The differences arise from small changes in DNA sequences, which produce new alleles and heritable variation. This is the raw material of microevolutionary change.

In this module you will focus on how new genetic differences are introduced into the gene pool of a population and how the likelihood of particular variations persisting in the population may change over time. The driving forces of environmental selection pressures are examined and natural selection is identified as the mechanism of evolution. Different modes of selection are explored through examples of natural selection.

VARIATION AND SELECTION PRESSURE

Microevolution refers to the small-scale variation of allele frequencies within a species or population, in which the descendant is of the same taxonomic group as the ancestor (Module 9.1). All organisms evolve by acquiring genetic change that enables populations to survive new circumstances. This results in adaptation.

There is always variation between individuals within a population. This is due to different combinations of alleles that have arisen from random mating, independent assortment and recombination during gamete formation and mutations. These factors, and the individual differences in gene expression and environmental factors, can lead to differences in phenotypes.

The conditions or factors that influence allele frequency in a population are known as **selection pressures** or **selective agents**. Selection pressures, together with mutation, are the driving force of evolution. Should a particular phenotype give an individual a survival advantage, that phenotype, and all the alleles that control it, is more likely to persist in the population. Selection pressures can be natural environmental pressures or artificial pressures brought about by humans through selective breeding.

A selection pressure is an environmental factor that affects the survival and reproductive success of an individual based on their particular phenotype.

Phenotypic variations in a population, combined with selection pressures, provide the driving force for evolution.

Natural selection

Natural selection is the influence of environmental pressures on allele frequency in a population. These environmental selection pressures affect the survival and reproduction of an organism by removing individuals through death or reducing reproduction rates. Individuals with the most advantageous phenotypes have an increased chance of producing fertile offspring.

Examples of environmental selection pressures include:

- climatic conditions, such as extreme temperature changes, flood and drought (Figure 9.2.1)
  - during drought, water supply is severely limited; plants that are dependent on local water and those lacking structures to conserve water are likely to die; plants with alleles conferring drought tolerance through structural or metabolic variations are more likely to survive and reproduce than plants without these traits
- competition for resources, such as the availability of food and water (Figure 9.2.1)
  - when food resources are limited, animals with genetically determined physical or structural adaptations that improve their ability to obtain limited food are likely to survive longer (have greater viability) and are more likely to reproduce (have higher fecundity) than animals without these adaptations
- competition for shelter
  - organisms with greater mobility to find shelter in extreme conditions, such as burrowing as protection from extreme cold, hot or dry conditions, are more likely to survive and reproduce than organisms without these characteristics
- mate availability
  - when mates are rare, reproduction and passing on alleles is decreased (reduced fecundity)
  - asexually reproducing species can be at an advantage when the conditions do not favour sexual reproducers, such as when mates are not available
- predator abundance
  - when predator numbers are high, prey species face increased survival pressure, that is, they are more likely to be eaten before reproducing (reduced viability)
  - when predator numbers are low, some prey species may reproduce faster than in usual conditions (higher viability and fecundity).

Environmental pressures influence allele frequencies of a gene pool due to a number of factors.

- Variation: There are genetic differences between individuals of a population.
- Viability: Survival is determined by environmental factors such as nutrient supply, temperature and water availability.
- Reproduction: Organisms can reproduce and alleles are heritable. The offspring are genetically similar (sexual reproduction) or genetically identical (asexual reproduction) to the parents.
- Fecundity: The rate of reproduction and number of offspring produced influences selection.
- Survival: Not all individuals are able to survive long enough to reproduce.
- Environmental selection pressures: Some phenotypes are better suited to the environmental conditions and give the individual a survival advantage over those of a different phenotype.

FIGURE 9.2.1 Drought can create competition for resources such as water and food.

When it comes to survival, some phenotypes (traits) cause individuals to survive better (greater viability) and reproduce more than others (greater fecundity). When the traits are determined by the allele combination in the organism, these useful alleles and useful traits aid survival in the presence of environmental selective pressures. Therefore, some phenotypes will give an individual an advantage over other individuals with a different phenotype, and the accompanying allele combinations for the favourable phenotype will be passed on through increased reproductive success. This results in adaptation of the population, as an accumulation of births and deaths cause a change in the frequency of phenotypes that are better suited to the environment that is applying the pressure on the population. This concept is often referred to as **biological fitness**, which refers to the ability of an organism to survive in its natural environment and reproduce.

FIGURE 9.2.2 *Banksia attenuata*, a native species of south Western Australia.

Alleles of the advantageous trait tend to increase in frequency in the gene pool, while alleles of the less advantageous trait tend to decrease. Modelling proposes that advantageous traits with a highly adaptive result in the population may persist in the population until many of the individuals possess them (close to 100% allele frequency). Over time, the population adapts to its environment.

Banksia attenuata is a woody shrub that survives the semi-arid, fire-prone environment of south Western Australia (Figure 9.2.2). Its adaptations include regulation of stomatal opening, altered metabolism and seed dispersal mechanisms that ensure its seeds are spread large distances to new locations. Unique base changes in several genes produce the alleles that have arisen in the population from *B. attenuata*'s adaptations to fire intervals, fluctuating rainfall and high temperatures. For example, variation in the *MDH* gene for the metabolic enzyme malate dehydrogenase, and the *S1P* gene controlling stomatal opening produce phenotypes that have a selective advantage in areas of variable rainfall and high temperature, thus enhancing survival (viability) of this species. The *AMPK* gene has a role in energy sensing and homeostatic adaptation to high temperature. Alleles of the *GEF* gene regulate homeostatic adaptations in plant morphology, including root growth, root hair differentiation, and development of flowers and vascular bundles. Genetic variation in this gene is linked to promoting regrowth after fire and is essential to survival of *B. attenuata*.

ALLELE FREQUENCIES IN A GENE POOL

As you learned in Module 5.2, the complete set of genes or DNA make up the genome of a species, and a species is defined by its particular genome. Yet the genome of individuals within that species will vary, depending on their unique combination of alleles and their non-coding portion of DNA. As a population changes over time through births, deaths and migration, and through natural and **artificial selection**, the percentage of individuals with particular alleles will also change.

All the alleles possessed by an entire population, which may potentially be passed on to the next generation, make up that population's **gene pool**. The relative proportion of a particular allele in a population is referred to as the **allele frequency**, and is often expressed as a percentage or a decimal (e.g. 25% or 0.25). The following equation shows you how to calculate an allele frequency.

$$\text{allele frequency} = \frac{2(\text{number of homozygotes}) + (\text{number of heterozygotes})}{2(\text{total number of individuals})} \times 100$$

When calculating the allele frequency of a particular trait, it is important to remember that homozygotes with the trait will have two copies of the allele, and heterozygotes will only have one copy.

For a variety of reasons, allele frequencies within a gene pool often change over time. For example, new alleles can occur as the result of genetic mutations, and the frequency of particular alleles can alter as a result of exchange with other populations, random shifts and changing environmental pressures.

Calculating allele frequency in the field

In the 1980s, blowfly resistance to the insecticide dieldrin was rising. Scientists investigated the influence of dieldrin in sheep dip and similar chemicals used for fly control at rubbish tips, and insect pest control in the region's fruit industry, in selection for the resistance allele. In the research they calculated allele frequencies. The phenotypes and genotypes are shown in Table 9.2.1. The resistance allele is the *Rdl* allele. The normal wild type allele at the gene locus is designated +.

TABLE 9.2.1 Phenotype (resistance to dieldrin), *Rdl* genotype and allele frequency in blowflies *Lucilia cuprina*

	Phenotype and genotype			Number of flies	*Rdl* frequency in the population
Location of blowflies	Resistant *RdlRdl*	Partially resistant *Rdl*+	Not resistant ++	*n*	
sheep dipped in dieldrin	20	50	58	128	0.35
rubbish tip where dichlorvos is used	23	28	9	60	0.62

Calculate the *Rdl* allele frequency for flies on sheep, using the following formula:

$$\text{allele frequency} = \frac{2(\text{number of homozygotes}) + (\text{number of heterozygotes})}{2(\text{total number of individuals})} \times 100$$

$$\begin{aligned} \textit{Rdl}\ \text{frequency} &= \frac{(2 \times 20) + 50}{2 \times 128} \\ &= \frac{90}{256} \\ &= 0.352 \end{aligned}$$

Calculate the *Rdl* allele frequency for flies at the tip:

$$\begin{aligned} \textit{Rdl}\ \text{frequency} &= \frac{(2 \times 23) + 28}{2 \times 60} \\ &= \frac{74}{120} \\ &= 0.617 \end{aligned}$$

The researchers concluded that flies carrying the *Rdl* allele survive better and have a selective advantage over wild type flies when dieldrin and dichlorvos are used in the environment.

Changing environmental pressure and allele frequency

Genetic variation within a population enables survival when new environments with new selection pressures change the allele frequencies of traits. This may lead to evolutionary radiation (Module 9.1). Charles Darwin recognised the similarities and the variations in the finches on the Galapagos Islands, and inferred patterns of common ancestry to explain the range of different species (Figure 9.2.3). The Galapagos Islands, when the finches first arrived, applied new selection pressures resulting in radiating evolution of different species.

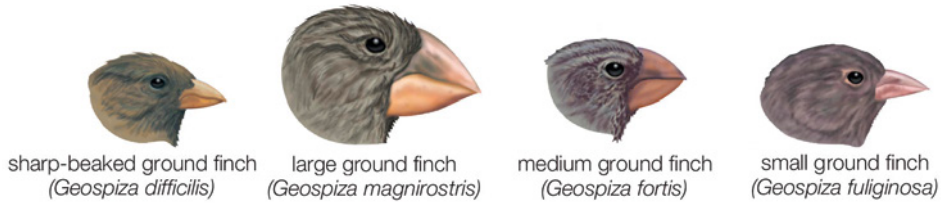


FIGURE 9.2.3 The ground finches of the Galapagos Islands were observed by Darwin and continue to be studied by scientists searching for the genetic basis of variation and evolution.

Scientists have collected data on many traits in the Galapagos finches and observed changes within species to investigate microevolution in response to environmental pressures. For example, the medium ground finch, *Geospiza fortis*, is a medium-sized finch with a medium-sized beak that lives on the ground, feeds on a range of seeds, and can also eat cactus fruit and arthropods. Beak size and depth were measured before and after a drought in 1977 (Figure 9.2.4).

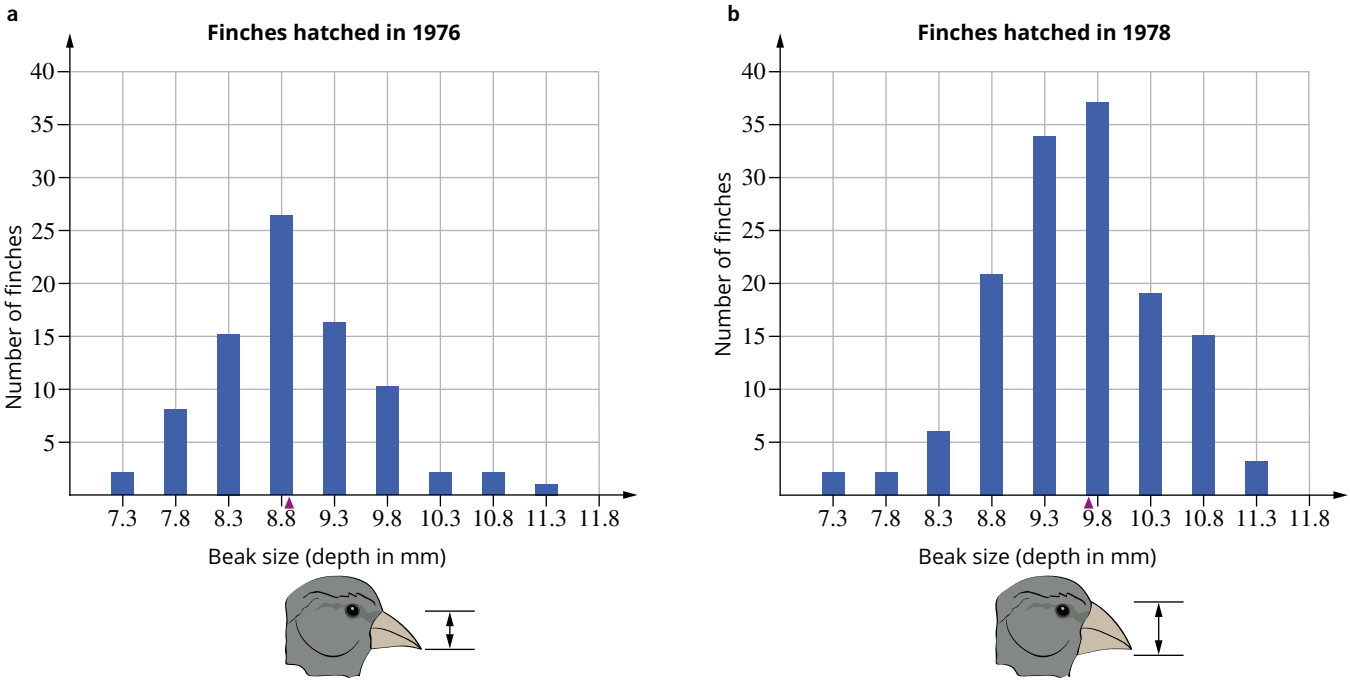


FIGURE 9.2.4 Beak depth measurements in *Geospiza fortis* chicks hatched (a) before and (b) after the severe 1977 drought on the Galapagos Islands. Average beak size is indicated by the purple arrow head. The increase in average beak depth enables birds to feed on larger seeds.

Average beak size increased after the drought. Whether the change in allele frequency is positive or negative, and whether the trait is advantageous, depends on the selecting agent. The seeds forming the bulk of the *Geospiza fortis* diet increased in size in response to the drought. A deeper bill was an advantage for feeding on larger seeds, and the selecting agent resulted in the decreased frequency of the smaller beak size phenotype. The deeper bill phenotypes had a competitive advantage for food and reproduced more successfully, changing the allele frequency in the population.

Recent DNA sequencing has identified genes associated with beak size. One strong candidate is a gene called *ALX1* that has two variants. The 'blunt' allele corresponds to blunt beaks (which are good for eating seeds) and the 'pointy' allele corresponds to pointy beaks (which are good for spearing insects). All of the blunt-beaked species, such as *Geospiza magnirostris*, were homozygous for the blunt allele. All of the pointy-beaked species, such as *Geospiza difficilis*, were homozygous for the pointy allele. The *Geospiza fortis* populations have a mixture of blunt and pointy alleles, which corresponds to the selecting agents of mixed food sources influencing the adaptation the populations.

New alleles arise by mutation

As you learnt in Chapters 2 and 5 and discussed in Module 9.1, some genetic variation between species and between individuals of the same species is a result of mutation. Mutations may affect a single gene, multiple genes or may involve entire chromosomes. The importance of mutation in evolution is whether it leads to a change in phenotype in a way that affects the organism's **viability** (the ability to survive in its environment) or **fecundity** (the rate of reproduction). Mutations may or may not lead to such phenotypic change.

- Mutations that do not change the amino acid sequence of proteins would have no effect on phenotype.
- Mutations that decrease the function of proteins might be detrimental for the organism. For example, mutations causing loss of function in enzymes for nutrient metabolism can lead to reduced viability due to lack of nutrition.
- Mutations that increase the production or activity of a protein can be beneficial, for example if it improves metabolism or helps the growth rate of offspring. However, overproduction of some proteins can cause them to aggregate (clump) in cells and tissues, leading to disease, and can decrease the likelihood of survival.
- Mutations can lead to phenotypic changes that limit the long-term survival of an organism but not affect its fecundity if the detrimental phenotype appears only after reproduction, as occurs in some inherited human diseases, such as Huntington's disease and Alzheimer's disease. Therefore, the mutant alleles can persist in the population.

Somatic mutations occur in body cells and only affect that individual. Germline mutations are heritable because they affect gametes and can therefore be passed on to offspring. A germline mutation may bring a new allele into a gene pool, potentially influencing the allele frequencies.

Genetic variation in a population can also arise through variation in chromosome number, such as **polyploidy**, in which organisms have extra full sets of chromosomes, such as a triploid (3 sets of chromosomes, $3n$) or tetraploid (4 sets, $4n$) karyotype (Module 7.1). This is common in plant varieties such as strawberries ($8n$), bananas ($3n$) and bread wheat ($6n$).

Genetic variation in a population can arise through new mutations and through variation in chromosome number, such as polyploidy.

Mutations occurring in regulatory sequences that control many other genes, such as developmental pathways, can create significant new variation, including new body plans.

An example of a mutation leading to microevolution is demonstrated by the yeast, *Cryptococcus neoformans*. One mutation that produces an early stop codon in the *tao3* gene causes the cells to change morphology from the normal ovoid budding cell type of yeast to an elongated cell, called a pseudohypha. This altered phenotype makes *Cryptococcus neoformans* resistant to phagocytosis by amoebae, its natural predator (Figure 9.2.5). This positive change in allele frequency produces yeast with increased survival against predators.

However, *Cryptococcus neoformans* is also a human pathogen, so researchers are studying the importance of these mutations and cell phenotype changes on the virulence of the yeast.

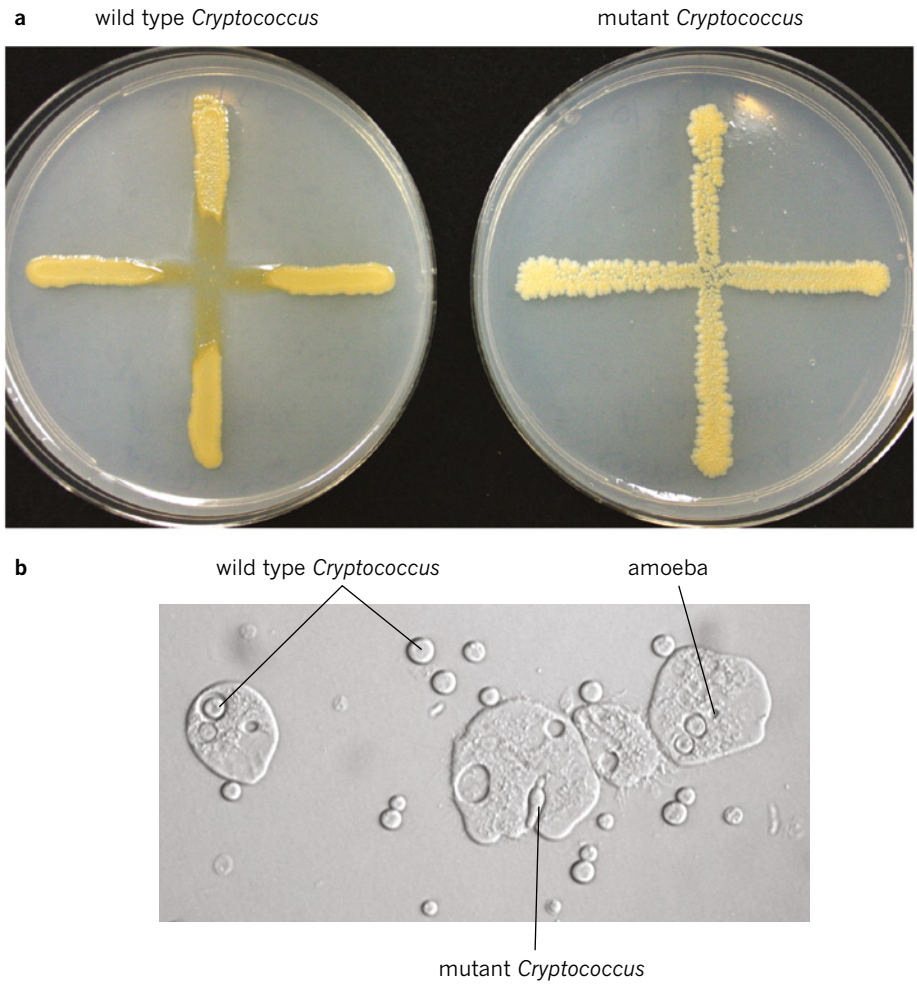
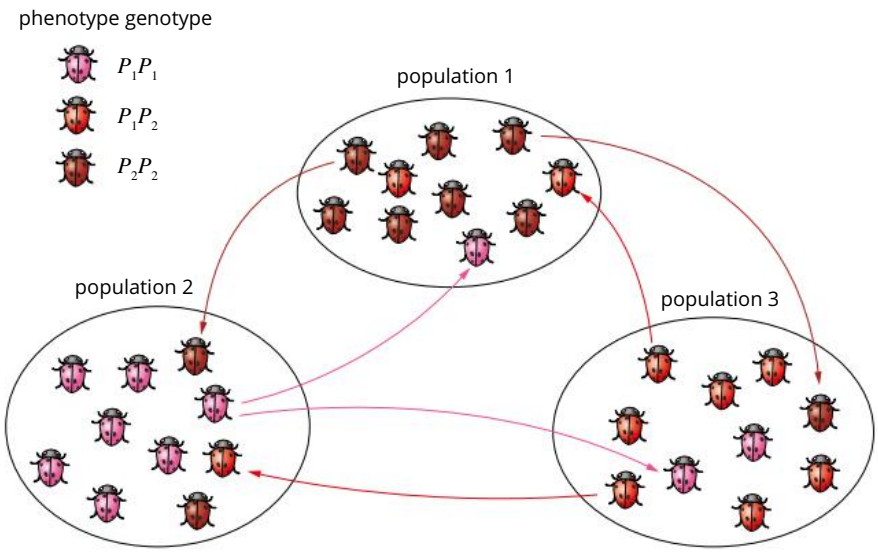


FIGURE 9.2.5 (a) *Cryptococcus* (the yellow cross pattern) growing on an agar plate: wild type (left) and mutant (right). Amoebae were dropped at the intersection of the cross. Wild type *Cryptococcus* are eaten and killed by amoebae (seen as the clear space at the cross on the left plate). (b) Microscopy of *Cryptococcus neoformans* and amoebae. Amoebas are much larger than *Cryptococcus* cells. Several ovoid wild type *Cryptococcus* cells have been consumed by phagocytosis and one mutant *Cryptococcus* is being engulfed by an amoeba.

Gene flow

Gene pools can change when new individuals join the population from a different gene pool or when some individuals leave a population. Such migration of individuals, and the alleles they carry, can result in **gene flow** (Figure 9.2.6).

When gene flow exists between two different populations, the gene pools may remain fairly similar. When gene flow is not possible between populations, the gene pools are said to be isolated. Genetic isolation is another key factor in the process of evolution and you will find out more about it in Module 9.3.



Seed dispersal in plants, interbreeding between different populations and migration can all result in gene flow.

FIGURE 9.2.6 Gene flow occurs by migration between neighbouring populations of a species. For three populations starting with one predominant phenotype and high frequency of one allele for a trait such as colour, gene flow introduces allelic and phenotypic variation into the populations.

WS 4.2.4

Genetic drift

Allele frequencies in a gene pool may also change randomly over time as a result of chance events. This is called **genetic drift**. Genetic drift is more clearly seen in small populations with little to no gene flow, as the random death of one individual can significantly alter the allele frequencies. Generally, in small populations, genetic drift results in the loss of genetic diversity over time as alleles are lost from the gene pool (Figure 9.2.7).

Genetic drift can occur when populations decrease for a period of time (a **bottleneck effect**) or in small founding populations, such as on isolated islands (the **founder effect**). The evolutionary significance of the bottleneck effect is explored further in Module 9.3.

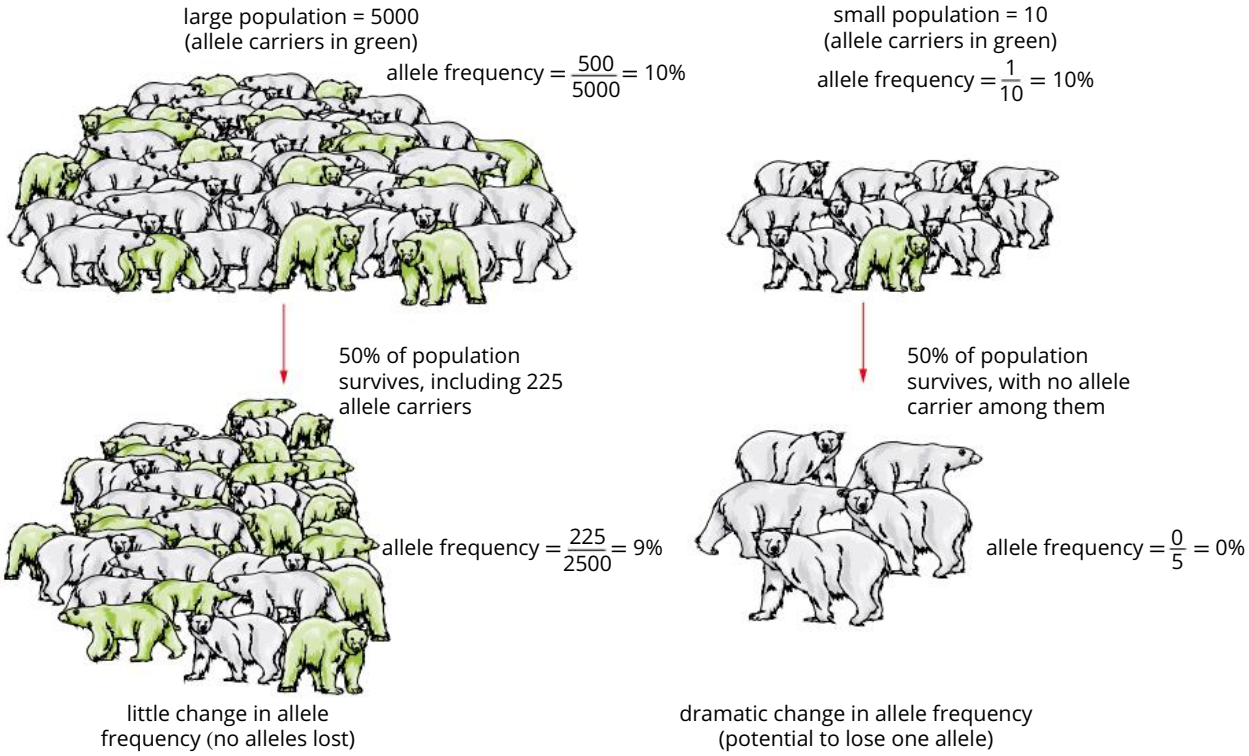


FIGURE 9.2.7 Genetic drift can cause greater changes in allele frequency in a small population than a large population.

TYPES OF PHENOTYPIC SELECTION

Selective pressures can work in favour of, or against, phenotypes and the alleles that generate the phenotype. In the case of the finches described (Figure 9.2.4), selection worked in favour of larger beaks.

Positive selection acts in favour of heritable traits. The allele frequency increases in the population.

Positive selection is selection that favours a heritable trait. As a consequence, the allele frequency for the selected trait is likely to increase in the population. With a strong positive selection pressure over many generations, an allele might become **fixed** in the population, meaning the frequency of that allele in the population reaches 100%.

International genome mapping and sequencing projects, such as the HapMap Project and the 1000 Genomes Project, provide DNA sequence and SNP data for identification, analysis and comparison of disease alleles, inheritance, genetic variation and adaptation.

Large international genome projects, such as the HapMap project, are identifying alleles approaching fixation in some human populations. One example of a gene showing signs of positive selection is a gene called *SLC24A5*, which is associated with skin pigmentation. Asian and African populations carry the ancestral gene. Genome scans identified a variant allele (called a derived allele) that causes a single amino acid substitution in the protein it encodes. This derived allele was present at 100% frequency in the European population but was absent or low frequency in the Asian and African populations (Figure 9.2.8).

FIGURE 9.2.8 Positive selection of alleles associated with skin pigmentation: ancestral allele (white); non-ancestral (derived) allele (caused by a mutation) (black). The frequency is represented as pie charts. A 100% frequency of the allele is shown as a filled circle. The derived allele is present at 100% in some European populations.

The high frequency of the *CCR5Δ32* allele in northern Europe before the emergence of HIV is not fully understood. It may have provided resistance to smallpox and been positively selected in some populations during historical smallpox epidemics.

Positive selection pressure is also seen for human immunodeficiency virus (HIV) resistance in humans. The emergence and spread of HIV in the 1980s placed significant selective pressure on human populations. Some northern European populations carried an allele, *CCR5Δ32*, that provided protection from infection. The normal *CCR5* gene encodes a co-receptor molecule expressed on the surface of white blood cells, which HIV uses to enter cells. A deletion mutation in this gene produces the allele *CCR5Δ32*, which codes for a protein that does not go to the cell surface, and the virus is not able to enter cells with this mutant co-receptor. Therefore, people who are homozygous for the *CCR5Δ32* allele are resistant to HIV infection and heterozygotes have some resistance to infection. Since the emergence and spread of HIV, the frequency of this allele has increased in geographical areas and populations where it was not previously found.

Negative selection acts against heritable traits. Allele frequency decreases in the population.

Negative selection disfavours a heritable trait. As a consequence, the allele frequency for the trait is likely to decrease in the population. The impact of negative selection depends on the dominant or recessive nature of the trait. For the simplest case of a characteristic determined by a single gene with two alleles, if the trait is recessive and appears only in homozygous recessive individuals, selection against the trait means the allele becomes less common and homozygote numbers decline. The heterozygotes are at an advantage because they show the dominant trait and their proportion in the population is likely to increase. If the trait is dominant, negative selection pressure will lead to a decline in the allele for the trait.

CASE STUDY 9.2.1

Positive and negative selection can depend on context

Viability can fluctuate depending on the selection pressures, so positive and negative selection may alternate. People with normal haemoglobin in their red blood cells have the genotype Hb^AHb^A. A mutation in the β-globin gene produces an allele Hb^S. People with the genotype Hb^SHb^S have sickle-cell anaemia. Abnormal haemoglobin produced from the Hb^S allele causes an abnormal sickle cell shape in red blood cells (Figure 9.2.9). Those affected by this illness have severely reduced aerobic capacity and often do not survive or pass on the alleles; that is, the Hb^S allele is selected against.

However, the Hb^S allele persists at quite high levels in several African countries where malaria is endemic. In these countries a significant proportion of the population are heterozygous and have sickle-cell trait, a less serious condition. Having one copy of the Hb^S allele provides a degree of resistance to malarial disease because the malaria parasite, *Plasmodium falciparum*, cannot enter and destroy the red blood cells as much as it usually does. Therefore Hb^AHb^S heterozygotes are at a survival advantage compared to Hb^AHb^A homozygotes living in a region with malaria.

Genomics and biomedical field research provide genetic data for the associations between disease phenotype and Hb^A or Hb^S allele frequency. The international HapMap project identified geographical regions where the Hb^S allele is present or absent and compared that to the incidence of malaria. Africa is one continent where malaria is endemic in many regions (Figure 9.2.10).

Another investigation in 2015 studied populations in villages in Gabon, Africa. Malaria is endemic throughout this region of Africa, and sickle-cell trait reaches as high as 28% of the population in Gabon. The prevalence of sickle-cell trait increased in villages with higher levels of malaria.

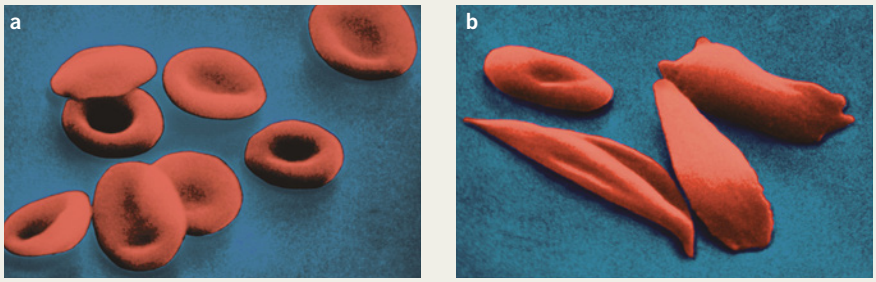


FIGURE 9.2.9 Viewed under the electron microscope there are various differences between the (a) normal and (b) sickle-cell red blood cell.

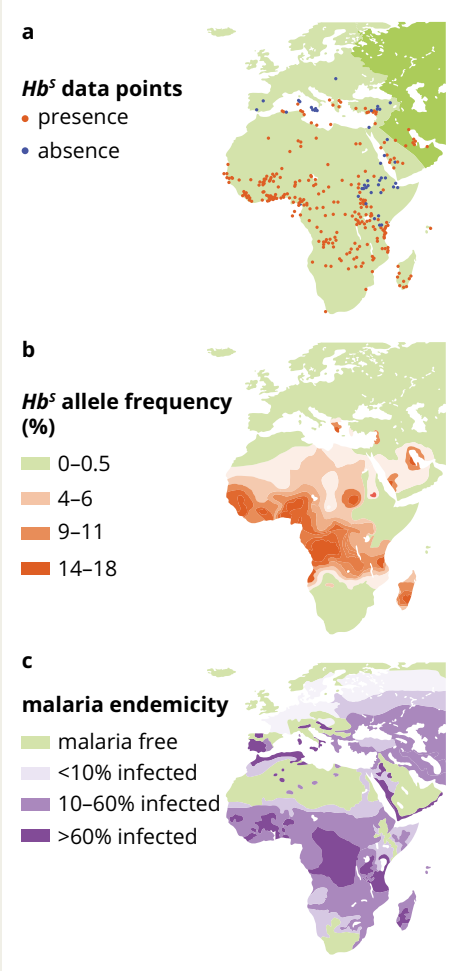


FIGURE 9.2.10 Hb^S allele and malaria in Africa. (a) Presence or absence of the Hb^S allele. (b) Frequency of the Hb^S allele. (c) Malaria endemicity: absent (green), occasional epidemics (pale purple) and endemic (darker purple shaded areas).

continued over page

CASE STUDY 9.2.1

Genotyping revealed that in villages with a high incidence of malaria, about 22% of the population (859/3958) has sickle-cell trait (Hb^AHb^S) (Table 9.2.2). The frequency of the Hb^S allele is high in this population compared to malaria-free populations, yet they found no individuals aged 17–80 with a homozygous $Hb^S\,Hb^S$ genotype. This confirms the high death rates of children with sickle-cell anaemia in these regions.

TABLE 9.2.2 Prevalence of Hb^A and Hb^S genotypes in relation to sickle-cell disease and sickle-cell trait in 17–80-year-old people

Genotype	Phenotype	Total number of individuals	Number not infected with malaria	Number infected with malaria
Hb^AHb^A	normal	3099	1492	1607
Hb^AHb^S	sickle-cell trait	859	405	454
Hb^SHb^S	sickle-cell disease	0	0	0
Total		3958	1897	2061

Review

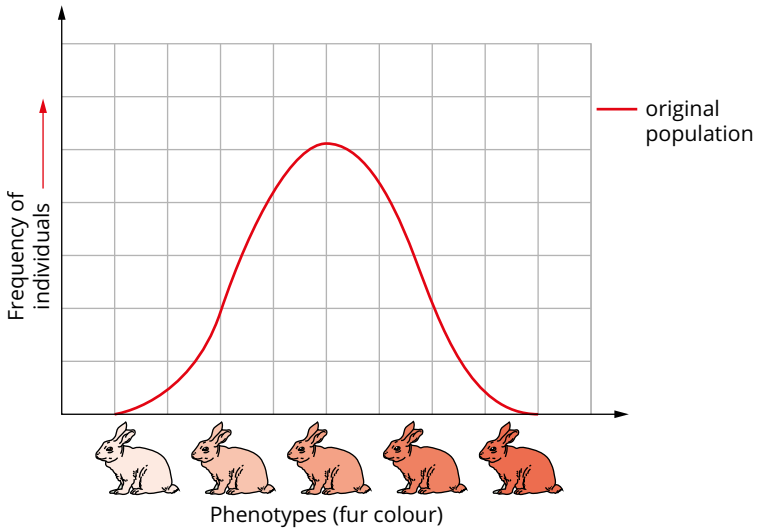
1. Deduce the type of selection acting on the Hb^S allele in:
   - **a** African populations with a high prevalence of malaria
   - **b** populations with no malaria
2. Calculate the percentage of non-infected people who are heterozygous for the *Hb* allele.
3. Calculate the chance of an individual with sickle-cell trait passing the Hb^S allele onto their children.
4. Infer the reason why there were no Hb^SHb^S heterozygotes identified in the populations analysed (17–80-year-olds).

Modes of phenotypic selection

Populations have a range of phenotypes for most traits, which shows a normal bell curve distribution (Table 9.2.3). When a selection pressure favours a particular phenotype, the frequency of the trait and the genes and alleles that control the trait, change over time.

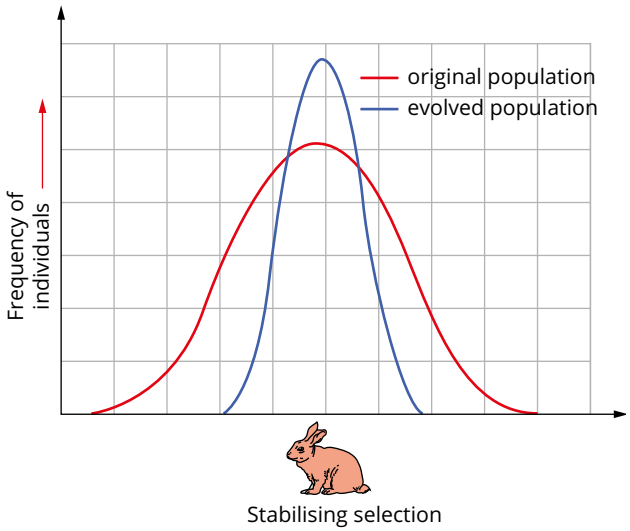
Three different modes of selection are stabilising, directional and disruptive. The original population has a range of phenotypes. A selection pressure may favour intermediate phenotypes (**stabilising selection**), a phenotype towards one end of the bell curve (**directional selection**) or extremes at each end of the bell curve (**disruptive selection**). Directional and disruptive selection are drivers of speciation and macroevolution. This is explored further in Module 9.3.

TABLE 9.2.3 Modes of natural selection in a population



Evolved populations

Stabilising selection

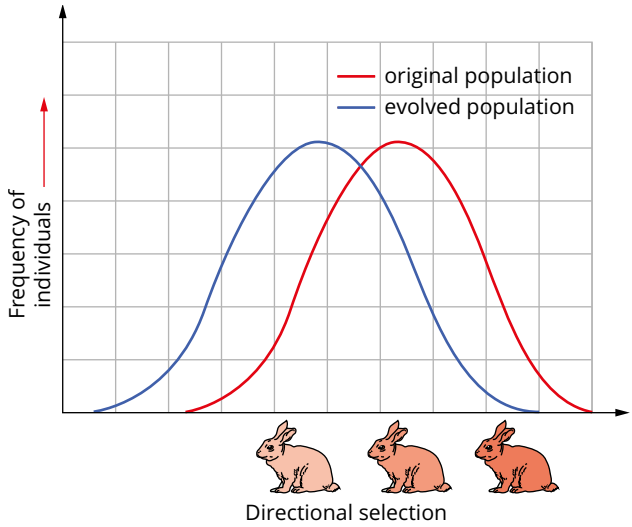
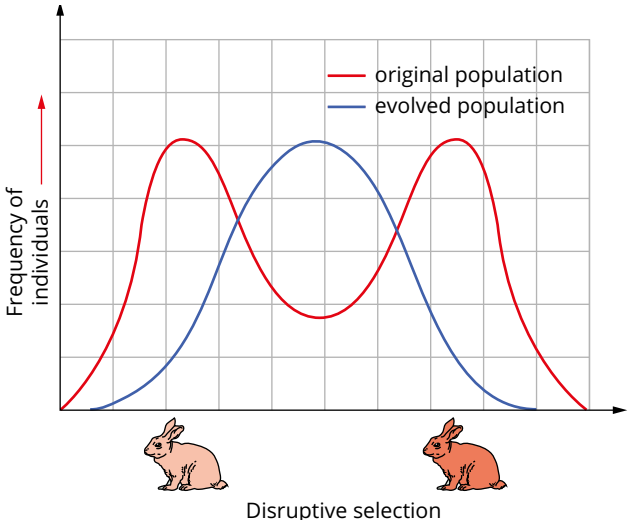


Selection favours intermediate traits in the phenotypic range and acts against extreme traits. Allele frequencies remain stable.

Stabilising selection maintains most traits in the normal distribution seen in their environment. Some examples are:

- Flowering can be maintained by stabilising selection, with those flowering too early or too late being less able to produce fruit.
- An optimum range of birth weight is maintained in animals. Infant mortality is higher in very low and very high birth weight babies.
- Pollinating insects can contribute to stabilising selection for flower size, to ensure the insects can reach the pollen.

Table 9.2.3 continued

Directional selection	
 Selection favours a particular phenotype and skews the allele frequency and phenotype in one direction. Allele frequency for the selected traits will increase in the population.	Directional selection is inferred in the following examples: • Beak size in Galapagos finches after drought: selection favours larger beaks so the mean beak depth increases (Figure 9.2.4 on page 456). • Herbicide resistance in weeds: a proportion of plants in a population may carry alleles that confer resistance to a herbicide. These plants have increased viability (survival) in the presence of the herbicide. Their proportion in the population will increase. • Heavy metal tolerance in plants: heavy metal contamination of environments, such as lead contamination, selects for the small proportion of plants with tolerance to the heavy metal. Their proportion in the population will increase. • Antibiotic resistance in bacteria: the use of antibiotics in medicine and agriculture selects for bacteria carrying resistance genes. Resistance genes arise by mutation and are transferred through clonal cell replication. Resistance genes on plasmids can be transferred between different bacteria (Figure 9.2.14 on page 467). • Pesticide resistance in insects: the use of insecticides in agriculture and livestock management, and insect vector control in public health management, has led to the evolution of insecticide-resistant insects. • Toxin resistance in bacteria: bacteria growing in environments with toxic compounds evolve resistance genes through mutation and horizontal gene transfer them between species (Figure 9.2.15 on page 468).
Disruptive selection	
 Selection favours extremes in the phenotypic range. The allele frequency for the selected traits may not change in the whole population. Genetic diversity may increase as extreme phenotypes are favoured. For traits controlled by multiple alleles and genes, alleles for intermediate traits could be lost, while the proportion of homozygotes is likely to increase.	Disruptive selection is inferred in the following examples: • Stickleback fish inhabiting lakes in British Columbia, Canada, derive from a common ancestor. Small phenotypes specialise in feeding on plankton while larger phenotypes specialise as benthic (near the sea/lake bed) feeders. This feeding behaviour is passed on genetically, but the two forms can interbreed. • Crossbills are small birds that feed on conifer cones. Their lower bill crosses under the upper bill. In some birds it crosses under on the left, in others on the right. Each phenotype has an advantage for extracting seeds from cones in different orientations.

EXAMPLES OF MICROEVOLUTION

Over many years researchers have recorded microevolutionary change evident as morphological, biochemical, genetic or behavioural characteristics. Researchers now make use of genomic technologies to add detailed genetic information to their field observations of species adaptation. Genomics can even provide evidence of evolutionary change in the absence of data on populations, such as microbes that cannot be grown and studied in a laboratory, but their DNA can be sequenced from environmental samples.

The following are examples of microevolution based on evidence for selection pressures acting to change allele frequencies.

CASE STUDY 9.2.2

Positive selection in high-altitude Tibetan populations

At high altitude, where the partial pressure of oxygen is lower (below 13 kPa) than sea level (around 20 kPa), the diffusion of oxygen into the blood occurs at a slower rate, which can cause altitude sickness. However, when people migrate to high-altitude areas, they can develop physiological changes, such as increases in haemoglobin and red blood cell numbers, enabling them to carry more oxygen. This can be detrimental because increased red blood cell numbers cause thickening of the blood. These physiological changes disappear on return to lower altitude. Such changes are not genetically determined and are not passed on to their children. Therefore, these physiological changes are not evolutionary adaptations.

In contrast, people and animals that have existed at high altitude for thousands of years have experienced positive selection over generations. The population has evolved adaptations to the lower partial pressures of oxygen (Figure 9.2.11). Tibetan people survive at high altitude by using oxygen more efficiently despite also having lower levels of haemoglobin and red blood cells, which avoids the blood thickening problem. Scientists have been searching for the genes and alleles selected in these adaptations to high-altitude life. It is expected there will be a change in allele frequency in the Tibetan population for haemoglobin and blood physiology, which are heritable traits, compared to sea-level populations.

FIGURE 9.2.11 Tibetan people and yaks are genetically adapted to high-altitude life.

Genome sequencing and scans for allele frequencies outside the normal range have identified several genes linked to high-altitude adaptation in Tibetans. In one study, researchers sequenced the exomes from 50 unrelated ethnic Tibetans living at around 4300 m altitude who all show heritable adaptation to the low oxygen (hypoxic) environment. They compared them to genome sequences of 40 Han Chinese and 200 Danish people. Han Chinese people showed a close genetic relationship to Tibetans but mostly live at low altitude without heritable physiological adaptations to high altitude. The Danish group are relatively genetically distant from the Tibetans, and live close to sea level.

The exome scans looked for SNPs, the single base changes in different alleles. The data was statistically analysed to compare the presence of the thousands of SNPs in the populations being studied. Figure 9.2.12 compares the SNP allele frequencies in Tibetans and Han Chinese. The graph shows that there are many alleles shared between Han and Tibetans (dots along the diagonal) and some alleles with very different frequencies.

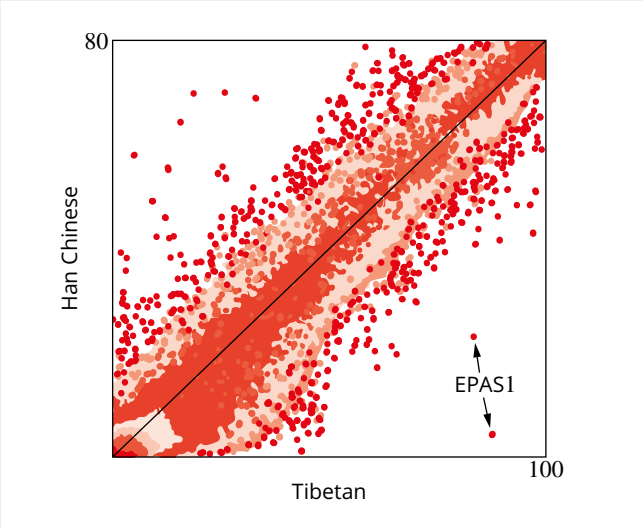


FIGURE 9.2.12 Comparison of SNP frequency between Tibetans (*x*-axis) and Han Chinese (*y*-axis). Each dot represents an allele. Most alleles are shared, shown by most dots located along the diagonal of the graph. The dots further from the diagonal are more likely to be found only in the Tibetan or Han population. Extreme outliers, like *EPAS1* in Tibetans, are interpreted as an indication of positive selection for the allele.

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CASE STUDY 9.2.2

Scientists look for allele frequencies well outside the normal range as indications of adaptations in a population. *EPAS1* (the outlier marked by the arrows in Figure 9.2.12) has a very high frequency in the Tibetan population but not in the Han or Danish populations. The *EPAS1* gene is known to code for a transcription factor associated with the production of red blood cells, and is activated when animals respond to low oxygen levels (hypoxia). The Tibetan allele correlates with the physiological adaptations to high-altitude living. Another study identified high allele frequencies of two other genes, *EGLN1* and *PPARA*, in Tibetans. These two genes also code for transcription factors whose expression is up-regulated in response to hypoxic conditions.

The researchers used the comparison of *EPAS1* in the Tibetan, Han Chinese and Danish populations to construct a phylogenetic tree (Figure 9.2.13).

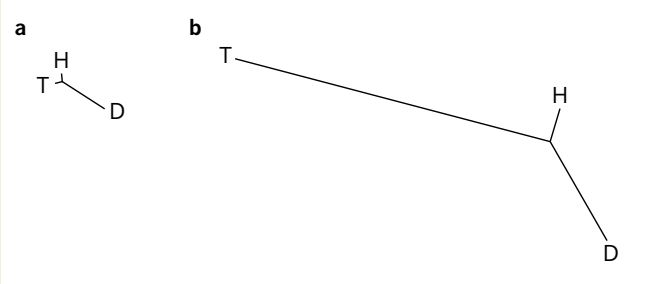


FIGURE 9.2.13 Phylogenetic tree constructed from *EPAS1* allele frequency in Tibetans (T), Han Chinese (H) and Danish (D) groups. (a) A phylogram comparing all alleles in the populations shows close genetic relationship between Tibetans and Han Chinese, and more distance to the Danish. (b) The phylogram constructed from *EPAS1* allele frequencies shows divergence of the Tibetans. These unrooted trees indicate the distance between the groups represented.

Review

1. The Han Chinese were included in the comparison because they are considered genetically similar to Tibetans. Explain how the graph of SNP alleles in Figure 9.2.12 (page 465) supports this similarity.
2. Explain why the study included data from the Danish population.
3. Describe how the phylograms in Figure 9.2.13 illustrate microevolution in Tibetans.

Microevolution in microbes

Bacterial populations tend to display rapid adaptation and microevolution because they:

- replicate rapidly (some replicate as fast as every 20 minutes)
- accumulate mutations
- share genes between variants of the same species
- can acquire genes from different species through plasmid transfer.

Selection for bacteria resistance to antibiotics

Bacterial populations have natural genetic variation in resistance to antibiotic drugs, due to the variations in genes for breakdown or removal from the cell (efflux or toxin removal) of cellular or environmental compounds. When bacteria are exposed to an antibiotic, the cells with a gene enabling resistance to that antibiotic are at a selective advantage and will survive, grow and pass on the resistance gene. This is microevolution occurring in bacteria, by means of positive directional selection for resistant phenotypes and resistance genes. Further mutation of these genes, followed by selection, leads to bacteria with greater resistance.

Antibiotic resistance genes can be located on the bacterial chromosome or on plasmids. Resistance genes are passed on through clonal cell replication and by bacterial conjugation, the process in which plasmids are transferred between bacteria of the same or different species. This is a type of horizontal gene transfer (Figure 9.2.14).

There are now many strains of bacteria resistant to the common antibiotics that have been used for many years, such as penicillin and erythromycin. For example, resistance to penicillin is produced by the *TEM-1* gene on a plasmid. The gene codes for an enzyme that degrades the penicillin, so bacteria with the gene are protected. Another example is resistance to erythromycin, which is prevalent in oral bacteria. Erythromycin kills bacteria by blocking the ribosome and inhibiting protein synthesis in the bacteria. Resistance is caused by *mef* genes, which code for proteins that pump certain compounds out of the cell (called waste pumps), and variants of the *erm(B)* gene, which codes for an enzyme that modifies the ribosome to make it resistant to erythromycin attack.

Stronger antibiotics, such as methicillin and vancomycin, have been used to treat resistant bacteria, but resistance to these antibiotics has also now evolved in bacterial populations.

Selection for bacterial resistance to environmental toxins

Some bacterial populations have evolved to survive and grow in the presence of high concentrations of toxins such as arsenic. Arsenic is a naturally occurring metalloid in soil and coal, forming compounds that are toxic at high concentrations. Bacteria with arsenic resistance genes have a selective advantage at a site in Pennsylvania where a coal seam fire has been burning since 1962, causing the release of arsenic from the coal and accumulation of arsenate and arsenite in the overlying soils.

Arsenic resistance in bacteria comes from enzymes that chemically reduce arsenate (reductases) or proteins in the cell membrane that pump arsenic compounds out of the cell (efflux pumps). These genes originate through mutation of existing enzyme genes which are used for specific toxins. The mutation results in an enzyme that is now able to process arsenic. They are inherited within a bacterial strain through clonal cell replication, and can also be transferred by horizontal gene transfer between bacteria, such as when plasmids containing resistance genes are transferred between bacteria (Module 8.2).

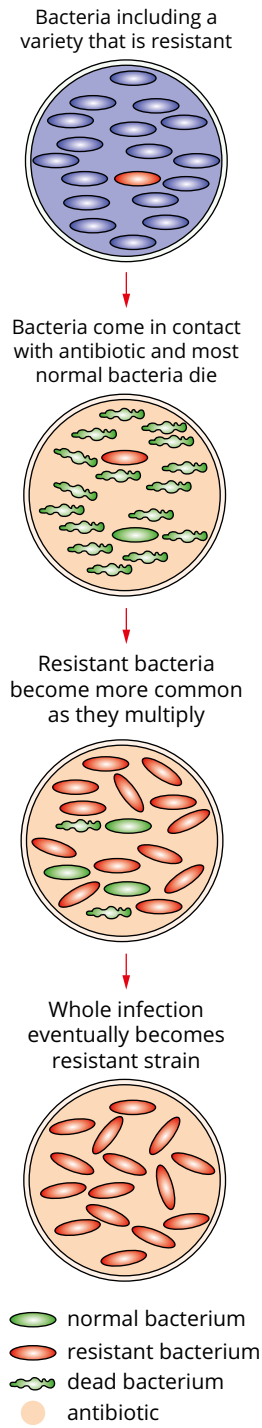


FIGURE 9.2.14 Antibiotic resistance genes originate by mutation. These genes may be transferred to other bacteria, often via plasmid transfer. In the presence of the antibiotic, the selective agent, microbes carrying the resistance gene survive and propagate a resistant strain.

Bacteria isolated from soils above the coal seam fire can grow with high concentrations of arsenic compounds in laboratory tests, providing evidence of directional selection for the resistant phenotype in the environment (Figure 9.2.15). The resistant phenotype is determined by different genes in various types of bacteria. Some bacteria carry a mutated gene for arsenate reductase enzyme, *arsC*. For example, *Bacillus* bacteria (strains 2–9) have the *arsC* gene and grow in high concentrations of arsenate and arsenite. Other bacteria carry a mutated gene for an efflux pump that allows cells to pump arsenic compounds out of the cell and prevent cell toxicity. For example, *Pseudomonas* (strains 23 and 24) has the *ACR3(2)* efflux pump gene and grows in very high concentrations of arsenate.

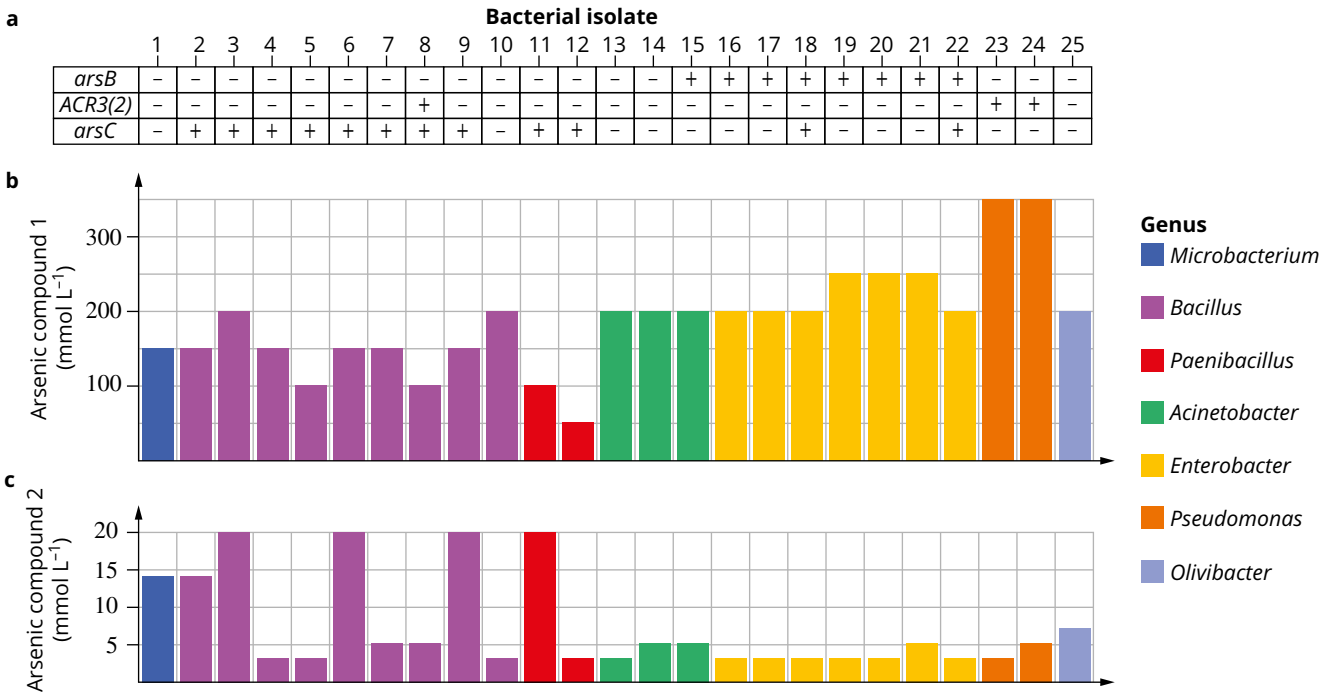


FIGURE 9.2.15 Arsenic-resistant genotypes and phenotypes of bacteria from arsenic-rich soils. (a) Genes for arsenic resistance present or absent (+/-); *arsB* and *ACR3(2)* code for arsenic efflux pumps, *arsC* codes for arsenate reductase. (b) and (c) Growth in arsenate or arsenite at concentrations up to 300 mmol L^{-1}. Different coloured bars represent different bacterial genera.

Evolution of drug resistance in parasites

Plasmodium falciparum, the parasite that causes malaria, has evolved resistance to the drugs used to control its spread. The parasite is transmitted to humans when they are bitten by an infected mosquito. The disease may also be passed between humans during blood transfusions. In humans, the parasite replicates inside red blood cells, causing them to burst. Symptoms include fever, vomiting and headaches.

The antimalarial drug chloroquine was discovered in 1934 and used in the 1940s. It was a safe and highly effective drug. However, in the late 1950s, chloroquine-resistant parasites emerged in Asia. A mine along the border of Thailand and Cambodia attracted many workers from neighbouring regions. As well as the environmental changes that occurred from the construction of the mine, which favoured the breeding of mosquitoes, workers were given inadequate doses of chloroquine. A mutation in the DNA of the parasite allowed the parasite to survive in the presence of chloroquine and spread to infect the many workers in the region, and then further afield as the workers migrated (Figure 9.2.16).

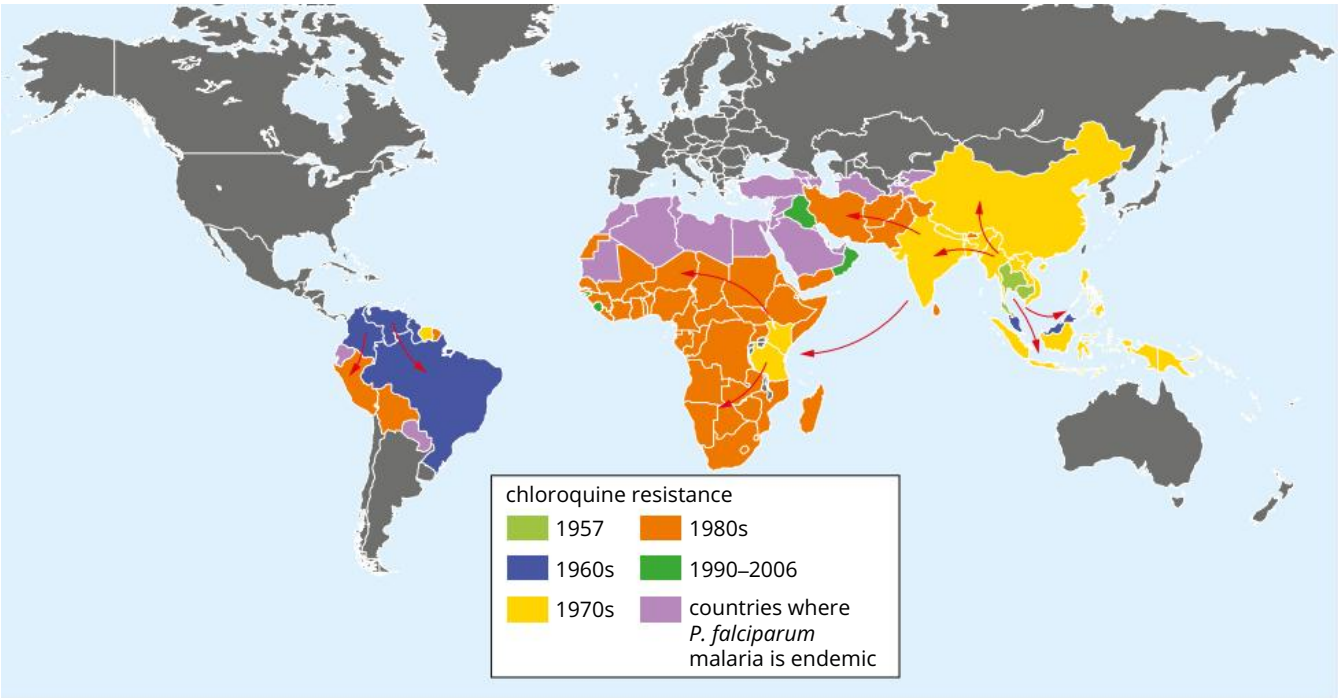


FIGURE 9.2.16 The history of chloroquine-resistant *Plasmodium falciparum*.

In the case of *Plasmodium falciparum*, the presence of chloroquine acts as a positive selection pressure, favouring parasite cells with the mutation. This resistance allele, *pfcrt*, codes for an efflux pump which expels chloroquine from the parasite. As resistance spread, chloroquine became an ineffective treatment.

Most sub-Saharan African countries, where malaria is endemic, stopped using chloroquine in the early 1990s. Genetic surveys in Kenya and Malawi identified a decrease in the frequency of the chloroquine resistance allele in *Plasmodium falciparum* after the countries banned chloroquine use. For instance, the frequency of the *pfcrt* allele declined in Kenya from around 95% to 60% between 1993 and 2006 (Figure 9.2.17).

This shows that when the selection pressure (chloroquine) is removed, the resistant parasites are no longer at a selective advantage, the positive directional selection ceases, and the frequency of the resistance allele declines. Over time, the parasite population will lose resistance and treatment of malaria with chloroquine can be resumed.

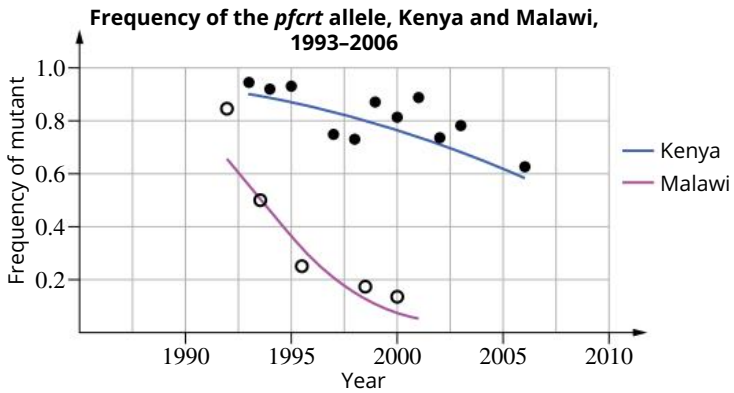


FIGURE 9.2.17 Decline in chloroquine resistance allele after discontinuation of the use of chloroquine in Malawi and Kenya.

Microevolution in viruses: New strains of influenza virus

The adaptation of viruses is rapid, often undergoing microevolution when infecting new hosts. Within hosts, viral genetic material may alter, resulting in the inclusion of a portion of the host DNA into their genetic material. This makes them more difficult for a host's immune system to detect them and allows them to evolve resistance to drug treatment. The change in the frequency of new gene variants in a virus can occur in a single host invasion.

FIGURE 9.2.18 Influenza virus undergoes microevolution when it infects new hosts. The virus is easily spread in water droplets released through coughing and sneezing.

Viruses with RNA genomes mutate faster than most organisms because RNA replication lacks the proofreading enzymes that check for mutations during DNA replication. Influenza viruses have an RNA genome with eight segments of RNA, which regularly undergo new mutations, particularly in the *H* and *N* genes used for entering and exiting cells. They can also generate new variants by a mixing and reassortment of the eight RNA segments between different strains when a cell is infected by more than one strain.

Flu virus circulates in populations of migratory birds, domestic poultry, pigs and humans. In humans, a new variant emerges almost every year and infects large numbers of people. This is called seasonal flu. New strains resulting from gene reassortment have new combinations of the surface antigens haemagglutinin (H) and neuraminidase (N). They may be more virulent and may also evade the host's immune defence, which was developed during previous infection with another flu strain. New flu strains arising in birds or pigs that gain the ability to infect human cells pose a high risk for pandemic flu.

Strategies for preventing the evolution of new pandemic flu strains include regular gene sequencing of human, bird and pig viruses to identify problem variants, culling of animals that are infected with dangerous new strains and limiting close interaction between humans and animals (Figure 9.2.18). Vaccinations before each flu season limits the number of infected people. When fewer people and other animals are infected with influenza virus, there is a reduced chance of genetic reassortment giving rise to dangerous new strains. However, as the virus evolves rapidly, new vaccines are required regularly.

The Victorian Infectious Disease Reference Laboratory in Melbourne is part of the World Health Organization Global Influenza Surveillance and Response System. It analyses influenza viruses circulating in the human population to help determine which viral strains should be included in the flu vaccine each year.

Microevolution in insects: Evolution of insecticide resistance

The Australian sheep blowfly (*Lucilia cuprina*) is the primary cause of flystrike in Australia and a major cost to the wool and sheep meat industries (Figure 9.2.19).

FIGURE 9.2.19 Australian sheep blowfly (*Lucilia cuprina*).

The major form of control is through the use of chemical insecticides by sheep dipping or jetting (spraying). Chemical control of blowflies from the 1950s to the 1990s included diazinon (see page 458 for discussion of gene flow) and dieldrin, which gave excellent control. However, blowfly populations evolved with insecticide resistance and effective control of the pest ceased.

Gene flow and microevolution

Gene flow between Australian sheep blowflies (*Lucilia cuprina*) is one way that chemical resistance spreads to fly populations not exposed directly to the chemical. *Lucilia cuprina* is the major cause of flystrike in Australian sheep. The fly lays eggs in wool and open wounds and maggots feed on the flesh. Diazinon was the most common chemical used to control flystrike until the 1980s, but the flies have evolved resistance. One study determined the frequency of the *Rop-1* diazinon resistance allele in flies captured on diazinon-treated sheep (using the sheep dip method) near Warrnambool in Victoria, and in a local rubbish tip where no chemicals were used for fly control.

Table 9.2.4 shows the allele frequencies in each population. *Rop-1* is the allele causing resistance to diazinon, and + is the wild type allele at the locus that does not confer resistance to diazinon. Heterozygotes show intermediate resistance.

TABLE 9.2.4 *Rop-1* genotype and allele frequency in *Lucilia cuprina* from sheep and rubbish tip

	Genotype			*Rop-1* frequency in the population
	Rop-1Rop-1	*Rop-1+*	*++*	
sheep	33	26	7	0.697
rubbish tip	3	31	26	0.308

The high incidence of the *Rop-1* diazinon resistance allele in the sheep population is evidence of natural selection, where genetic variation in flies with a mutation conferring diazinon resistance are at a selective advantage when sheep are treated with diazinon. The flies survive and breed, and the allele frequency in the population increases. The presence of the *Rop-1* resistance allele in the fly population at the rubbish tip indicates gene flow, because diazinon was never used at the tip. Mating between sheep and tip populations can spread the resistance alleles.

Genetic resistance to dieldrin

Resistance to dieldrin is determined by a single gene, with two alleles R^R and R^S.

- Flies with genotype $R^R R^R$ are highly resistant to dieldrin.
- Flies with genotype $R^S R^S$ are susceptible and are killed by the insecticide.
- The heterozygote $R^R R^S$ has a resistant phenotype intermediate to those of the homozygotes.

The R^R allele was at a very low frequency in the sheep blowfly population before the introduction of dieldrin. There is evidence that when dieldrin is not present, $R^R R^R$ and $R^R R^S$ individuals are less viable than $R^S R^S$ individuals. Flies with the R^R allele do not survive as well or leave as many fertile progeny as $R^S R^S$ genotypes. The R^R allele will be selected against if dieldrin is not being used. This negative selection of R^R results in microevolution towards an increased frequency of the R^S allele.

In contrast, when dieldrin is used, resistant phenotypes are now at a selective advantage and the R^R allele undergoes positive selection. This is reflected by the rapid increase in the proportion of dieldrin-resistant phenotypes in sheep blowfly populations after dieldrin was introduced for blowfly control (Figure 9.2.20).

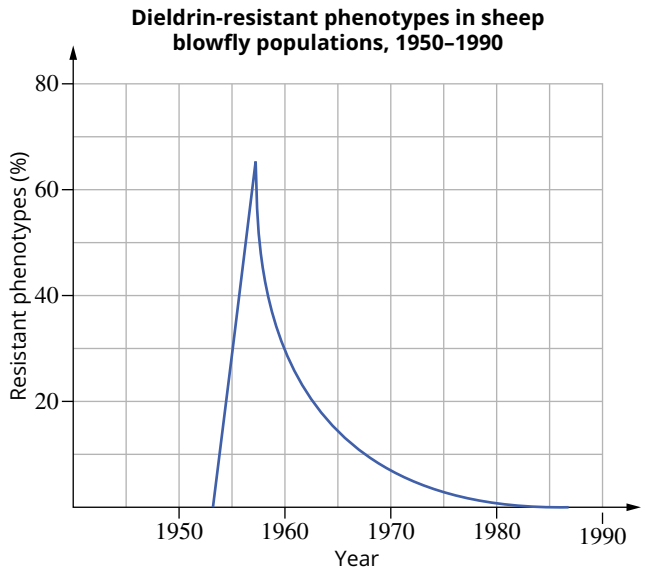


FIGURE 9.2.20 The change in the percentage of dieldrin resistance in the Australian sheep blowfly following the introduction (1955) and removal (1958) of dieldrin for blowfly control.

In 1958 a new pesticide, unrelated to dieldrin, was introduced. It killed many of the dieldrin-resistant phenotypes. In the absence of dieldrin, the frequency of the R^R allele and the dieldrin-resistant phenotypes in the population declined because flies susceptible to dieldrin were again at a selective advantage relative to resistant flies.

In the laboratory, scientists exposed flies to different concentrations of dieldrin to investigate the effect on the frequency of the dieldrin resistance allele (Figure 9.2.21). In the fly populations grown with the highest dieldrin concentration, the resistance allele reached 100%, that is, it became fixed in the population. This indicates selection for the resistance allele at high concentrations of dieldrin. In the flies grown without dieldrin, the frequency of the resistance allele did not increase. Therefore, when dieldrin is present at high concentrations, flies with the dieldrin resistance allele survive, reproduce and pass on the resistance allele to their offspring.

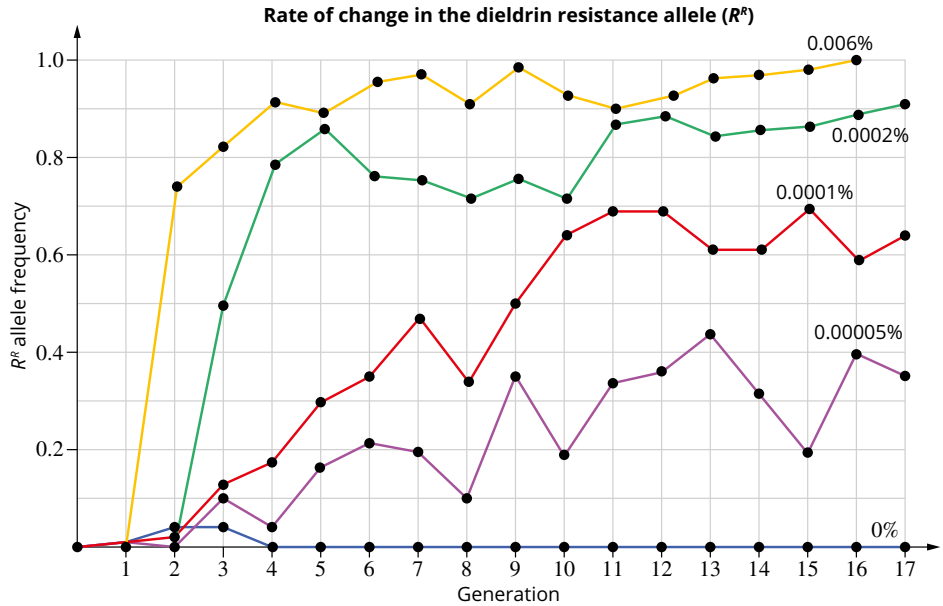


FIGURE 9.2.21 The experiment started with populations with the dieldrin resistance allele (*Rdl*) frequency of 1%. Different fly groups were exposed to different dieldrin concentrations (0–0.006%) for 17 generations.

Dieldrin is still used as an insecticide in some countries, where it is selecting for resistance alleles in insects.

CASE STUDY 9.2.3

Selection in the rock pocket mouse

Positive selection for colouration is seen in the American rock pocket mouse, which inhabits a region spanning northern Mexico, New Mexico and southern Arizona. The mice have two different hair colours. The majority of mice have light, sandy-coloured hair and inhabit light-coloured granite hills and desert sands (Figure 9.2.22). In some regions, where there are dark-coloured lava flows, dark-haired mice are found.

FIGURE 9.2.22 Rock pocket mouse, wild type or light phenotype (*dd*), on light rock.

Predators of this mouse include owls, rattlesnakes, foxes and coyotes (Figure 9.2.23). Colouration of the mouse is an important protection from these visual predators, especially owls, which have a visual advantage from the air.

FIGURE 9.2.23 Owls are major predators of the rock pocket mouse. They can see a light mouse on dark rock even when hunting at night.

Researchers identified the *Mc1r* gene as the genetic basis for the colour difference. *Mc1r* codes for melanocortin 1 receptor and controls skin and hair pigment production in mammals. Rock pocket mice have two alleles for the *Mc1r* gene: *D* and *d*. Mice with genotype *dd* have light-coloured hair. This is considered the wild type genotype. Mice with genotypes *DD* and *Dd* have dark-coloured hair, called a melanic phenotype. Researchers sequenced the alleles and identified the mutations that result in four amino acid differences in the proteins produced. Compared to the wild type *d* allele, the *D* allele produces a more active protein, resulting in more melanin pigment production.

Field observations showed a strong association between mouse phenotype and the rock colour of their habitat. The evidence indicates selection for dark phenotype in mice inhabiting the dark lava flows.

The researchers hypothesised that positive natural selection is the mechanism for the presence of two mouse hair colour phenotypes in the granite and lava rock habits. They sampled animals from six locations—three granite and three lava flow areas—along a transect of 35 km through a national wildlife refuge in Arizona. The mice were trapped, measurements taken and data recorded by field researchers, then they were released. The pooled data are shown in Table 9.2.5.

TABLE 9.2.5 Data collected of mice that were trapped by researchers

Ground type and colour	*N*	Phenotype (fur colour)	Number of mice	Proportion of population (%)
granite/sand light brown (3 sites)	168	light hair	120	
		dark hair	48	
lava fields dark grey/ black (3 sites)	57	light hair	3	
		dark hair	54	

Review

1. Copy and complete Table 9.2.5 by calculating the proportion of each phenotype at each location. Give your answers to one decimal place.
2. Describe the association between the melanic phenotype and ground colour.
3. Table 9.2.6 shows the genotype data from the Pinacate lava flow site (dark lava ground).

TABLE 9.2.6 Genotype–phenotype associations between *Mclr* alleles (*D*,*d*) and coat colour in at the Pinacate site

		Genotype		
		DD	*Dd*	*dd*
mouse phenotype	light	0	0	12
	dark	11	6	0

Use this equation to calculate the following:

$$\text{allele frequency} = \frac{2(\text{number of homozygotes}) + (\text{number of heterozygotes})}{2(\text{total number of individuals})} \times 100$$

   a. frequency of the *D* allele in light and dark mice at this site
   b. frequency of the *d* allele in light and dark mice at this site
4. State whether the *Mc1r* mutant allele *D* correlates with mouse hair colour.
5. Identify the most significant selection pressure(s) or selecting agent(s) acting on the population. Describe how the selection pressure leads to the allele frequency seen at the Pinacate lava flow site.

9.2 Review

SUMMARY

- Natural variation exists between individuals of the same species because many genes have multiple alleles that are present in the population in different frequencies.
- Allele frequencies are a measure of how common a particular allele is in the gene pool of a population, and are typically expressed as percentages or decimals.
- Mutation is a source of new alleles in a population.
- Allele frequencies change in response to natural selection or genetic drift.
- Natural selection is the influence of environmental pressures on allele frequencies of a population, which occurs because of genetic variation between individuals, and the survival and reproduction of those individuals with favourable phenotypes (traits).
- Phenotypes that are better suited to environmental selection pressures have higher adaptive values than those that are less suited. Individuals with alleles associated with the phenotype are more likely to survive and reproduce. These alleles are more likely to persist in the gene pool and increase in frequency over time.
- Positive selection favours a heritable trait, leading to increase in allele frequency for the trait.
- Negative selection acts against a heritable trait, leading to decrease in allele frequency for the trait.
- Gene flow is the movement of alleles into and out of a gene pool. It can occur when different populations interbreed or individuals migrate between populations.
- Genetic drift is the change in allele frequencies in a population due to chance or random events. This is most likely to affect small populations. The bottleneck effect and founder effect are types of genetic drift.
- Stabilising selection favours intermediate traits in a phenotypic range and maintains allele frequencies.
- Directional selection favours a particular phenotype and shifts allele frequency in one direction.
- Disruptive selection favours extremes in the phenotypic range. Allele frequencies may not change but proportions of homozygotes may increase.
- Evidence for natural selection comes from field observations of changes in populations.
- Genomics, DNA and RNA sequences add new evidence for natural selection and allow the identification of the specific genes and alleles involved in evolutionary change.

KEY QUESTIONS

Retrieval

1 Define natural selection.

2 Describe three key factors that contribute to natural selection.

3 Describe ways that gene flow can occur between two populations.

4 Define positive selection and describe the possible effect on allele frequency in a population.

Comprehension

5 Calculate the allele frequency for the purple allele, in a population of 150 individuals with 80 being homozygous for purple flowers, 40 homozygous for white flowers and the remaining 30 individuals being heterozygous purple/white.

6 Red-bellied black snakes are predators of cane toads. Describe the kind of evidence that would indicate biological evolution of red-bellied black snakes in relation to toads, rather than short-term physiological change or learned behaviours.

7 Explain why genetic drift may have more impact on a small population than a large one.

8 Compare the effects of positive and negative selection on allele frequencies in a population.

Analysis

9 Infer the reasons why conditions, such as hip dysplasia in dogs, are more common in selectively bred populations than in wild populations.

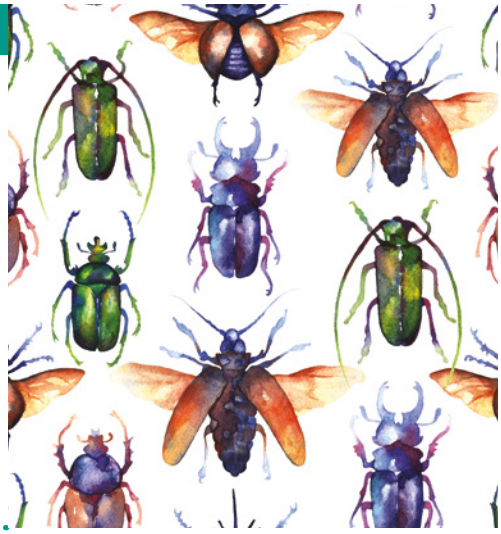
10 Compare gene flow and genetic drift as mechanisms for changing allele frequencies in populations.

11 A population of flowering plants has three scent phenotypes in the flowers: strong scent, medium scent and mild scent. Pollinator insects tend to be repelled by the strong scent and spend most time visiting milder-scented flowers. Over time, a change in phenotype frequency to a milder-scented flower was observed. Identify and describe the mode of selection acting on the population.

9.3 Speciation and macroevolution

BY THE END OF THIS MODULE, YOU SHOULD BE ABLE TO:

- recall that speciation and macroevolutionary changes result from an accumulation of microevolutionary changes over time
- identify that diversification between species can follow one of four patterns: divergent, convergent, parallel and coevolution
- describe the modes of speciation: allopatric, sympatric and parapatric
- understand that the different mechanisms of isolation—geographical (including environmental disasters, habitat fragmentation), reproductive, spatial and temporal—influence gene flow
- explain how populations with reduced genetic diversity (i.e. those affected by population bottlenecks) face an increased risk of extinction
- interpret gene flow and allele frequency data from different populations in order to determine speciation.



Evolution is the change in the genetic composition of populations over time. This can be observed as changes in allele frequencies in a population over time. Genetic change in populations of an ancestral species can lead to new species (Figure 9.3.1). In the previous modules you learnt that natural selection acting through environmental pressures leads to microevolution, the changes in allele frequency within species. In this module, you will explore the evolutionary processes that form new species and the different patterns of change in macroevolution.

A species is a group of organisms that can interbreed to produce viable offspring. Viable offspring survive to maturity and are fertile.

Macroevolution is the variation of allele frequencies at or above the level of species over geological time, resulting in the divergence of taxonomic groups, in which the descendant is in a different taxonomic group to the ancestor.

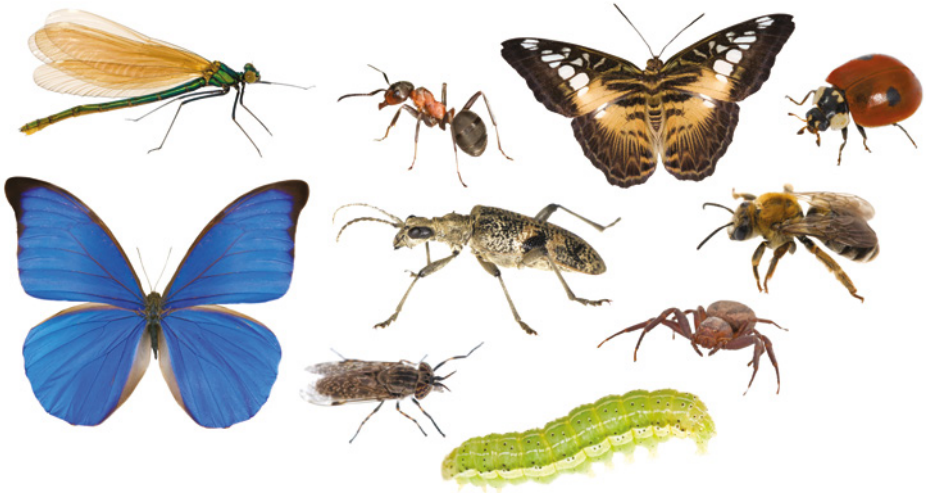


FIGURE 9.3.1 There are close to 1 million known insect species on Earth, with new species discovered every year. The diversity seen in insect species is largely due to different selection pressures over a long period.

PROCESSES OF SPECIATION

As you learned in the previous module, natural selection is a driving force of evolution. Some alleles will increase or decrease in frequency, depending on their effect on the viability (survival) and fecundity (reproductive success) of the organism. Environmental conditions change over time, altering the environmental selective pressures on a population. Allele frequencies change through gene flow and genetic drift and in response to natural selection. Eventually the population may change so significantly through microevolutionary changes that it is deemed a new species. This is macroevolutionary change—the result of an accumulation of microevolutionary changes over time.

Speciation is the formation of a new species following an event that splits a lineage, such as geographical, morphological, behavioural or reproductive isolation of populations.

FIGURE 9.3.2 Koalas from different parts of Australia are able to interbreed to produce viable fertile offspring and thus are considered the same species.

Speciation is the evolutionary process by which a lineage separates and forms new species.

FIGURE 9.3.3 The zony is a hybrid individual resulting from the interbreeding of a zebra and a donkey.

It is important to remember that populations and species evolve, not individuals. Changes in the gene pool are the evidence of evolution, not mutations within an individual.

The relationship between changing environmental pressures and evolution of a species can be seen in ecosystems that have experienced little change over time. If the environment remains unchanged, the traits of organisms will continue to have high adaptive value, and there will be little change to allele frequencies over time.

Defining species

As you learned in Chapter 2, a species is defined as a group of individuals that are genetically similar enough to produce fertile **viable offspring** when interbreeding (Figure 9.3.2). In microevolution, the changes in allele frequencies within a species produce genetic variation. If members of variant populations can still breed together and produce viable fertile offspring, we consider them members of the same species.

A species can also be defined as a genetically isolated population. **Genetic isolation** occurs when there is no gene flow between two populations, and so the species' gene pools are isolated from each other. When a lineage is split and enough microevolutionary change has occurred so that individuals of the separate groups can no longer breed and produce fertile offspring, they are considered separate species. This is the process of speciation.

ISOLATING MECHANISMS

Isolation of individuals or groups from a population through space or time can result in the separation of a lineage. This is often a starting point for speciation. Mechanisms such as **geographical isolation** and **temporal isolation** limit gene flow between populations. Over time, allele frequencies change in the new populations due to genetic drift. New and different mutations appear in separated populations and eventually new species originate. Once new species form, these isolation mechanisms maintain the separation and usually prevent interbreeding between species.

Genetic isolation of one species from another can be a result of several mechanisms, and sometimes one or more at a time. These mechanisms can be broadly categorised as prezygotic (occurring before reproduction) or postzygotic (occurring after reproduction).

Prezygotic isolating mechanisms typically prevent individuals from different populations from interbreeding. In other words, they prevent fertilisation from occurring. Commonly, these mechanisms prevent mating. Sometimes they act after mating takes place by preventing gametes from fusing and forming a zygote.

When interbreeding between individuals from different species does result in offspring, they are called hybrids (Figure 9.3.3). Isolating mechanisms that occur after reproduction are those that typically prevent a hybrid from developing into a fertile adult, such as **hybrid inviability** or **hybrid sterility**.

The different forms of isolating mechanisms are summarised in Table 9.3.1.

TABLE 9.3.1 Summary of isolating mechanisms

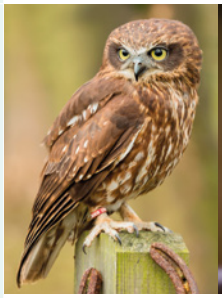
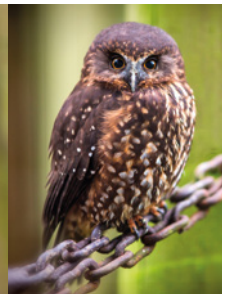
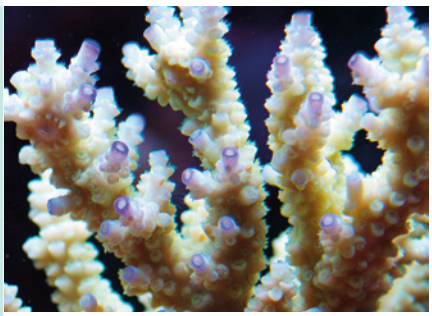
Isolating mechanism	Consequence	Examples	
Before reproduction			
Spatial isolation Any physical dimension over an area of any size (includes examples such as geographical isolation, habitat fragmentation and distance).	Reduces gene dispersal over an area (of any size of interest) and increases genetic drift within populations. Gene flow is reduced and overall, genetic diversity decreases within populations and results in divergence between populations.	For the Australian bush rat, a distance of over 1 km has shown to result in reduced gene dispersal. Studies show clusters of genetic similarity among individuals within 1 km. Gene flow is reduced and genetic divergence occurs.	Australian bush rat
Geographical isolation separates populations by physical and geographical barriers, such as oceans, deserts, mountain ranges, glaciers and long distances.	Reduces access to individuals in other populations for breeding, therefore limiting the gene pool.	The southern boobook is an Australian owl that is genetically distinct from the New Zealand owl, morepork. One reason that they are genetically isolated is that the Tasman Sea separates them.	 Australia's southern boobook  New Zealand morepork
Habitat fragmentation partitions habitats and reduces the range of individuals, potentially creating localised populations.	Decreases genetic diversity, decreases gene flow across populations and increases genetic drift.	The damming of the Queanbeyan River near Canberra, and the translocating of hundreds of Australian Macquarie perch adults upstream beyond a waterfall, resulted in habitat fragmentations and also separation from the spawning habitat. Now all populations of the Australian Macquarie perch have very little genetic diversity.	Juvenile Australian Macquarie perch
Temporal isolation is a misalignment of reproductive systems (e.g. fertility) between individuals or populations.	Reduces or excludes gene flow and can result in sympatric speciation.	Thirteen species of *Acropora* corals in Western Australia were investigated and it was discovered several species spawned in spring while the others spawned in autumn. Colonies are distinctly related to spawning events with genetic analysis showing gene flow to be non-existent, unless extreme climatic events cause early spawning in autumn for all species. Genetic analysis shows sympatric speciation.	 *Acropora tenuis*
Environmental disasters immediately reduce the number of individuals within a population or across populations depending on the size of the disaster.	Often causes a bottleneck which includes a reduction in genetic diversity and consequently genetic drift. Gene flow is temporally disrupted until populations regrow.	Among other mechanisms, bushfires have been most prevalent in the last century to bottleneck the Buxton gum to only two forest reserves near Buxton, Victoria.	Buxton gum

Table 9.3.1 continued over page

Table 9.3.1 continued

After reproduction		
Hybrid inviability occurs when hybrid offspring of different species are not viable, or show limited development and reduced viability, due to the incompatibility of genes from the different parental species.	Sheep and goats are in the same taxonomic family and subfamily, but are in different genera. They often mate when sharing pastures, but zygotes are typically non-viable and any resulting fetuses are usually stillborn. Occasionally hybrids survive, but are sterile.	
Hybrid sterility occurs when hybrid offspring of different species are sterile or show limited reproductive capacity due to failure of gamete formation. The lack of homologous pairs of chromosomes causes meiosis to fail, so gametes are not produced.	The mule is the offspring of a female horse ($2n$ = 64) and a male donkey ($2n$ = 62). The mule has 63 chromosomes in total. Hybrids like mules do not have homologous pairs of chromosomes because their genetic material came from different species. Without homologous pairs, meiosis cannot proceed normally and the gametes, if any are formed at all, cannot interact correctly in order for fertilisation to occur. Mules, like all hybrid offspring, cannot reliably reproduce.	Mules

Sexual selection

Sexual selection is one way in which behaviour, acting as a reproductive isolating mechanism, results in evolution. Most animals exhibit some level of sexual selection in which at least one sex selects their mate based on specific traits. Although it may appear that mates are chosen on the basis of an irrelevant characteristic, the chosen traits are often indicators of good health, strength and fitness. The alleles of these mates may then be inherited by offspring, depending on Mendelian inheritance.

Sexual selection is very common in birds. For example, barn swallows select mates based on the length of their tail streamers, which indicate health and fitness. Another example is the bowerbird, which selects a mate on the basis of the showiness and the particular structure and colour of the bower it builds by retrieving objects from the habitat (Figure 9.3.4). Again, this indicates the bird's health and fitness.

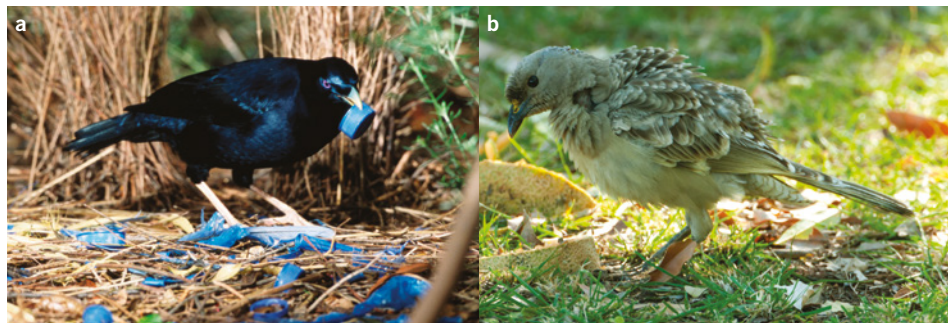


FIGURE 9.3.4 (a) Satin bowerbirds inhabit wet forests in the southeast of Australia and are attracted to blue objects. (b) Great bowerbirds occur in the tropical ecosystems of northern Australia and are attracted to white, green and red objects. Although the species are also geographically isolated, this difference in mate attraction is an example of sexual selection and behavioural prezygotic isolation.

Animals may compete with members of their own sex for mates of the opposite sex. Animals such as sea lions, antelope and kangaroos come into direct physical conflict over mates, usually resulting in a single male winning the right to mate with a large number of females. These conflicts demonstrate the individuals with the fittest phenotypes are the ones that are most likely to produce more offspring and healthier offspring after mating. In this way, the favourable alleles are more likely to be inherited by the next generation, which increases the frequency of those alleles in the gene pool over time.

MODES OF SPECIATION

Speciation is the evolution of new species from an ancestral species. All forms of speciation require isolating mechanisms to limit or prevent gene flow between populations. Different isolating mechanisms result in different speciation processes. Here we describe three forms of speciation: allopatric, sympatric and parapatric.

Allopatric speciation

The most common form of speciation is allopatric speciation. **Allopatric speciation** occurs when a geographical barrier divides a population (Figure 9.3.5). The spatial isolation prevents individuals of the separated subpopulations from interbreeding.

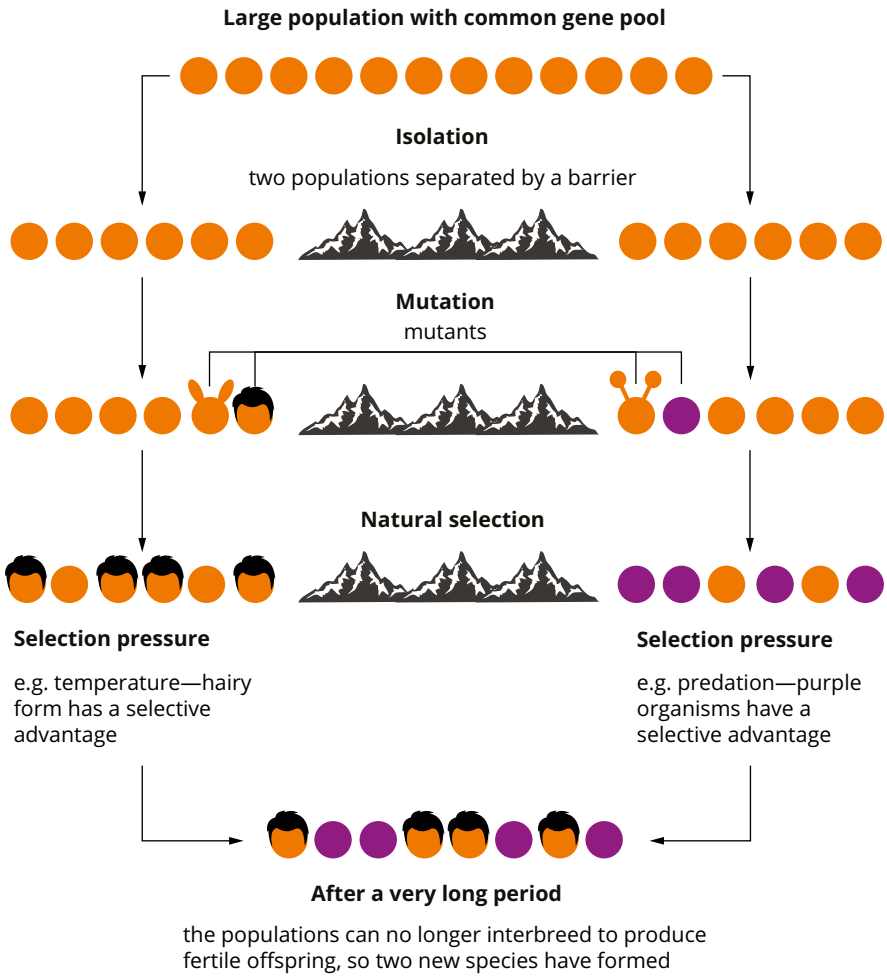


FIGURE 9.3.5 Geographical isolation leads to allopatric speciation.

FIGURE 9.3.6 Tasmania has a high number of unique species that are not found on the mainland, such as the leatherwood.

Over time, different environmental selection pressures, including genetic drift, drive change in the allele frequencies of the two subpopulations. Eventually, the two subpopulations may diverge genetically and morphologically to the point where they can no longer successfully interbreed should they come into contact again. They may become two distinct species.

Australia has a number of distinct regions of biodiversity that are isolated geographically by water, mountain ranges and deserts. For example, the south-west corner of Western Australia has a very high number of endemic species (native to a specific area or region), both plant and animal, due to the geographical isolation of the central arid zone. Tasmania also has high levels of endemism because of its separation from the Australian mainland by Bass Strait (Figure 9.3.6).

Allopatric speciation in the coast banksia

The coast banksia, *Banksia integrifolia*, is one of the largest and most common banksias on Australia's east coast. It grows as far north as Mackay in Queensland and as far south as Port Phillip Bay in Victoria. Over this extensive geographical range, populations vary in the shape of their fruits and leaves (Figure 9.3.7).

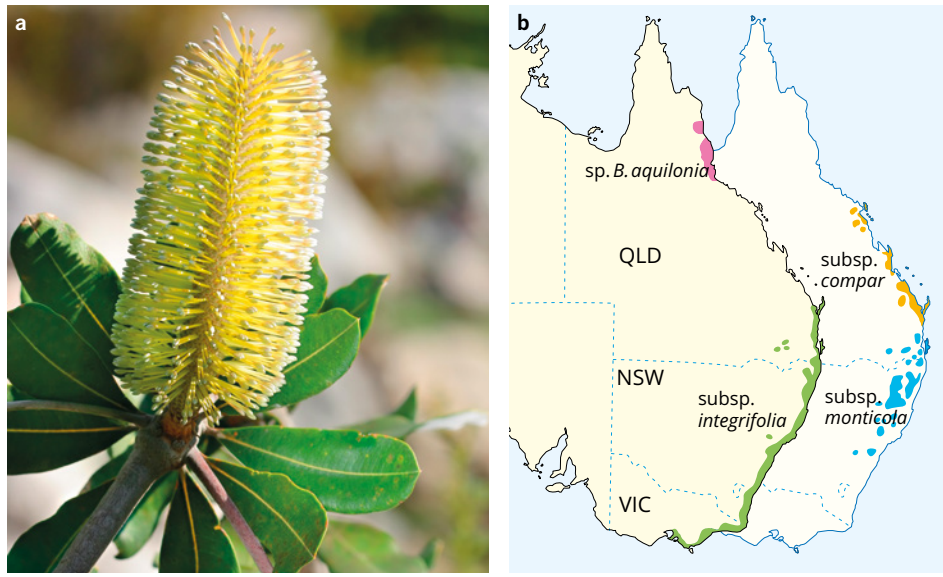


FIGURE 9.3.7 (a) *Banksia integrifolia*. (b) Distribution of different forms of coast banksia.

Four forms have been identified. Plant taxonomists have recognised three of these forms as varieties or subspecies of *Banksia integrifolia*. The fourth form, *Banksia aquilonia*, was originally identified as another subspecies but is now recognised as a related but distinct species. Geographically, *Banksia aquilonia* is separated from the *Banksia integrifolia* subspecies by more than 200 km. This distance prevents reproduction between *Banksia* plants, leading to isolated populations that accumulate different genetic variations and speciation.

A molecular study using a DNA fingerprinting technique (amplified fragment length polymorphism or AFLP) confirmed that the three *Banksia integrifolia* subspecies are genetically distinct from one another and different from *Banksia aquilonia*. The technique also confirmed that plants with a leaf shape intermediate between subspecies are the result of gene flow between populations. The distributions and characteristics of the species and subspecies of coast banksias are summarised in Table 9.3.2.

TABLE 9.3.2 Distributions and characteristics of species and subspecies of coast banksia

Species and subspecies	Distribution	Form
B. integrifolia subsp. compar	• scattered distribution • southern Queensland and north-eastern New South Wales • coastal	• large glossy adult leaves • seedling leaves elliptical • small straight side teeth
B. integrifolia subsp. monticola	• south-eastern Queensland and north-eastern New South Wales • montane regions above 650 m in altitude	• narrow adult leaves • seedling leaves obovate (widest at the top) • large teeth with curved sides
B. integrifolia subsp. integrifolia	• southern Queensland, New South Wales, Victoria • coastal	• leaves shorter than *B. integrifolia* subsp. monticola • seedling leaves obovate • curved
B. aquilonia	• north-eastern Queensland • coastal	• long narrow adult leaves • largest fruit • fringe of stiff hairs on the midrib, underside of the leaf

Sympatric speciation

Speciation can occur even when geographical barriers do not isolate populations. This is called **sympatric speciation** and is often a result of disruptive selection (Module 9.2). Sympatric speciation is more common in plants than in animals and it can occur in two ways.

Most habitats contain microhabitats: small areas with highly specific environmental conditions. When part of a population occupies a microhabitat, the difference in selective pressures can be enough to drive sympatric speciation. In this situation, the two populations become genetically isolated by temporal or behavioural isolating mechanisms. Molecular analysis of such populations is likely to indicate divergence in allele frequencies, when the proportions of alleles differs between populations of the species, but sometimes indicate that gene flow continues during, and perhaps after, the speciation process.

Sympatric speciation in plants more commonly occurs through polyploidy (where every chromosome in a cell has more than two copies). Polyploid offspring can be produced by chromosomal mutations, errors in meiosis or hybridisation. Polyploid plants can still reproduce successfully through self-pollination or vegetative reproduction. However, a polyploid plant is unlikely to successfully reproduce with its diploid counterparts and so sympatric speciation by polyploidy is instantaneous.

Sympatric speciation on Lord Howe Island

Researchers have studied sympatric speciation of the flora on Lord Howe Island, a very small isolated volcanic island 600 km east of Australia (Figure 9.3.8 on page 482). Lord Howe Island is of great interest because many endemic species evolved there, but it is so small it is considered impossible to have true geographical isolation between populations on the island. Therefore, other forms of isolation or unusual pathways to speciation may be at work. The closest land is Balls Pyramid, a small mountain 24 km away, and the distance to other landmasses limits gene flow with related species on these distant lands that can pollinate via wind.

The researchers used molecular phylogeny to determine genetic relatedness and variation between the island's plant species. None of the plants was polyploid and the DNA evidence indicated that gene flow was occurring, which is rare in speciation. The researchers concluded that sympatric speciation was driven by flowering time and altitude of these new species.

Lord Howe Island is also well known for its VIP insect, the very important Lord Howe Island stick insect. This large stick insect is a phasmid that was once common on Lord Howe Island, but populations plummeted when rodents hitchhiked to the island on sailing boats. It was thought to be extinct, but a few somehow travelled to Balls Pyramid and are now in a breeding program at Melbourne Zoo.

Lord Howe Island phasmid

FIGURE 9.3.8 Lord Howe Island. The island's geographical isolation leads to sympatric speciation. Diverging species have overlapping geographical range and may speciate while gene flow continues.

Parapatric speciation

Parapatric speciation is rare. **Parapatric speciation** occurs where populations are not geographically isolated, but where there is significant variation in habitat conditions within the range of the original population. Gene flow is possible between the two populations but, in a large population with a large range, individuals are more likely to breed with nearby individuals. Mating is not entirely random. Slight differences in environmental pressures from one end of the range to the other can result in localised variation in allele frequencies within the population. Over time, this can result in two distinct species.

The three modes of speciation are compared in Figure 9.3.9.

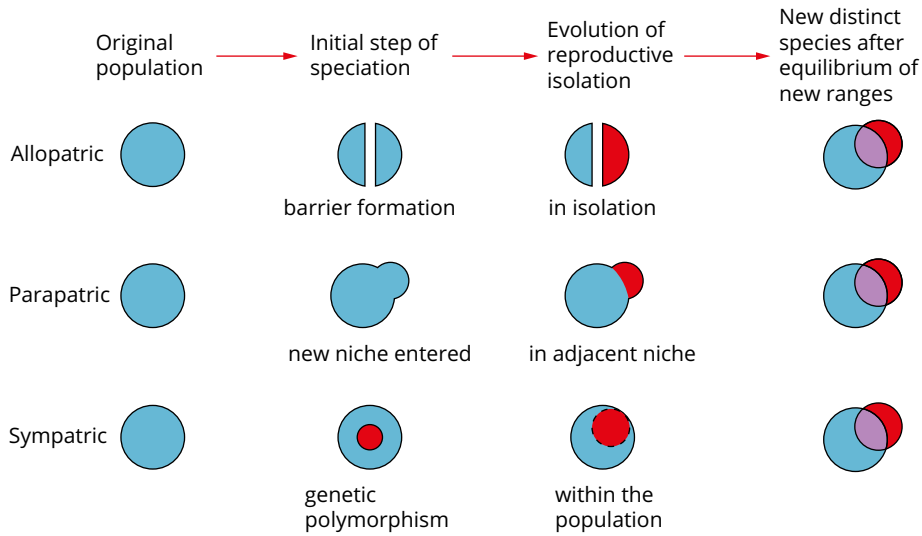


FIGURE 9.3.9 Comparison of three modes of speciation.

CASE STUDY 9.3.1

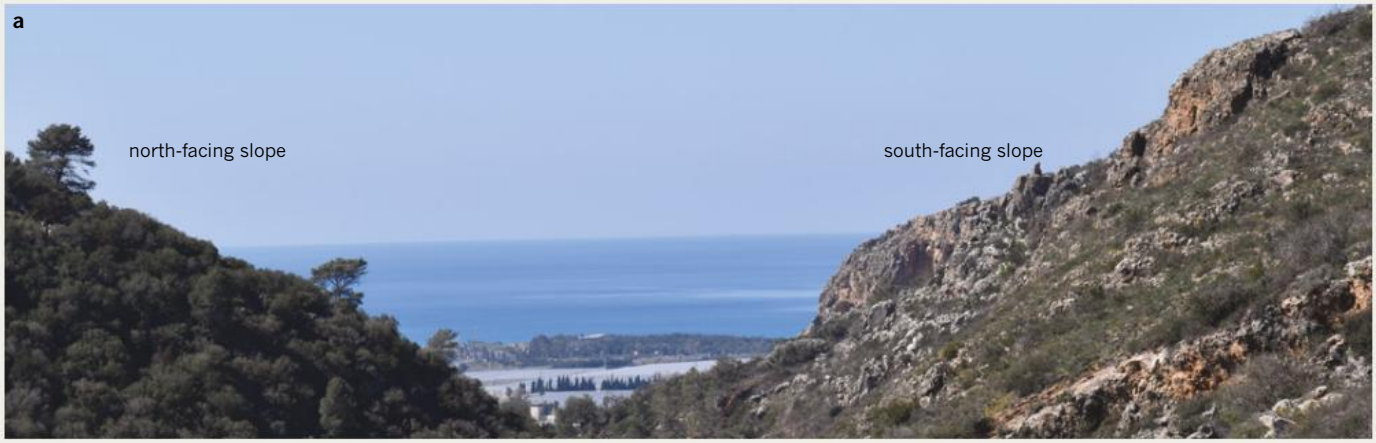
Isolation within a canyon: *Drosophila* on the verge of speciation

A canyon in Israel, aptly called Evolution Canyon, is home to the fruit fly *Drosophila melanogaster*. The canyon spans only 100–400 m but it has very different microclimates on each side (Figure 9.3.10a). Different populations of *D. melanogaster* inhabit the north-facing and south-facing slopes (Figure 9.3.10b). Although the flies are capable of travelling across the canyon, they maintain separate populations on either side of the canyon and exhibit marked differences in body size, tolerance to heat and desiccation, among other divergent characteristics. They also prefer to mate with flies from the same slope.

Researchers have been studying adaptation in these populations. One measure is genetic distance (D). If two populations have many alleles in common, they have a small genetic distance, but if the alleles are less common then there is greater genetic distance, presuming divergence (Table 9.3.3 on page 484). Two identical populations have a D value close to zero. A D value > 0.15 represents a large genetic distance. The *D. melanogaster* populations 15 km apart in the Congo have a large genetic distance, but the *D. melanogaster* populations only 400 m apart in Evolution Canyon show an even greater genetic distance.

Researchers also mapped microsatellites (non-coding short repeat DNA regions) to study genetic variation and gene flow. As microsatellites are found on chromosomal DNA, they are inherited in the same way as genes.

Genetic differentiation (F_{ST}) is another genetic measure that uses microsatellites. The distance between microsatellite regions can show the degree of difference between populations. This measure also indicates whether the populations reproduced with each other in the past. A large interbreeding population has zero genetic differentiation, but when a population becomes isolated and gene flow declines, more alleles become fixed in the



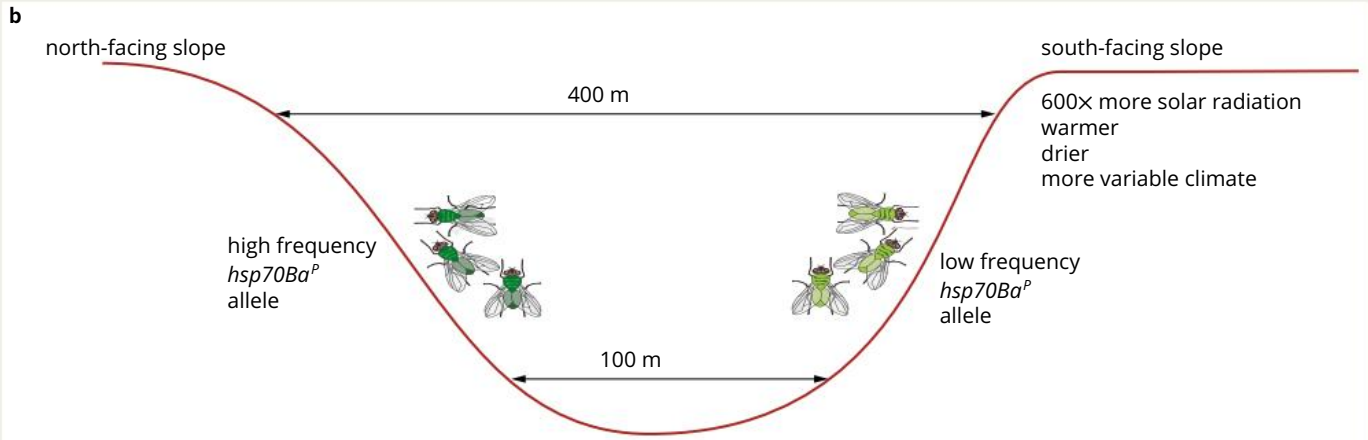


FIGURE 9.3.10 *Drosophila melanogaster* in Evolution Canyon are a model population for studying evolution. (a) Evolution Canyon in Israel. (b) *Drosophila melanogaster* populations occupy different slopes of the canyon, which have different microclimates.

continued over page

population (Module 9.2) resulting in genetic distance, yet genetic differentiation can continue. The genome becomes more 'differentiated', so the F_{ST} value increases (Table 9.3.4).

F_{ST} values are on a scale of 0–1:

- 0 means low differentiation (populations freely interbreed)
- > 0.25 means a high degree of differentiation
- > 0.5 means very high degree of differentiation
- 1 means populations are completely separate with no gene flow.

Tables 9.3.3 and 9.3.4 show the result of these analyses for the Evolution Canyon populations and other *Drosophila* species or populations.

TABLE 9.3.3 Genetic distance between *Drosophila* populations or species

Population or species comparisons	Genetic distance (D)
D. melanogaster in Evolution Canyon (north-facing slope vs south-facing slope)	0.566
D. melanogaster vs *D. simulans*	0.5–0.6
D. melanogaster (two populations, 15 km apart, Congo)	0.44

Table 9.3.3 shows that the genetic distance between the Evolution Canyon populations, D = 0.57, is similar to the genetic distance between completely different species, *D. melanogaster* compared to *D. simulans*. Despite being only 400 m apart, they show greater genetic distance than two populations separated by 15 km in the Congo.

The Evolution Canyon flies also had a much higher genetic differentiation score (0.36) than the same species in other locations, meaning they are more genetically isolated and have little gene flow with other *Drosophila* populations, including the ones across the canyon. These data together suggest that the Evolution Canyon fruit fly populations are on the way to becoming separate species.

TABLE 9.3.4 Genetic differentiation in populations of *Drosophila melanogaster*

Species	Population	Microsatellite differentiation (F_{ST})
D. melanogaster	Evolution Canyon (north-facing slope vs south-facing slope)	0.361
D. melanogaster	California, Zimbabwe, Kenya	0.121

The researchers also analysed gene sequences. One locus of interest is a regulatory region for the heat shock protein (hsp) 70Ba, which has a normal allele and a variant allele. The north-facing slope flies had almost 30-fold higher frequency of the variant allele, indicating very limited gene flow between the populations. Therefore, although these populations occupy the same canyon, they are genetically isolated. The researchers conclude that, while some gene flow occurs, these populations are close to being separate species. They propose the microclimates on the north and south slopes of the canyon are driving adaptation of the populations and speciation.

Review

1 In the *Drosophila* populations, the *hsp70Ba* gene has a variant allele named *hsp70Ba*^{P}. For simplicity, we will call the normal allele *A* and the variant allele *P*. Table 9.3.5 lists the number of individuals analysed and their genotype.

TABLE 9.3.5 Genotype data for *hsp70Ba* locus in *Drosophila* in Evolution Canyon

Population	Number of individuals analysed	Homozygous (*PP*)	Heterozygous (*AP*)	Heterozygous (*AA*)	Frequency of *P* allele
north-facing slope	96	5	55	36	
south-facing slope	124	0	3	121	

a Copy and complete the table. Calculate the frequency of the *P* allele in each population.
b Calculate the ratio of this allele in the north-facing slope and south-facing slope populations.
c Identify the evidence leading the researchers to conclude that 'gene flow is low but not zero'.
d The researchers conclude that, 'The *D. melanogaster* case presented here, however, is unique not only in detecting massive fine scale genetic differentiation in a very mobile organism but in revealing genetic changes associated with developing speciation driven by adaptation to local environments.'
Infer the specific type of speciation the researchers identified. Justify your choice.

PROCESSES IN MACROEVOLUTION

Accumulation of microevolutionary changes and speciation are the basis for macroevolution. Macroevolution is the variation of allele frequencies at or above the level of species over geological time, which gives rise to new taxonomic groups. This means that the same type of microevolutionary processes, such as changes in allele frequency and adaptation, occur over a longer geological timescale. It includes speciation, the factors that drive adaptive radiation, selection within larger taxonomic groups, species going separate paths (divergence) or appearing similar (convergence), as well as mutations in developmental pathways and the origins of new body plans.

When different species originate from a common ancestor, they diversify as they continue to accumulate genetic variation through mutation and genetic drift. Different mutations and selection pressures drive divergence. In some circumstances, environmental selection pressures lead to genetically distinct species sharing similar morphology or behaviours, in other words, their appearance or behaviour converges. Here we outline four different patterns of species diversification: divergent evolution, convergent evolution, parallel evolution and coevolution.

Divergent evolution

Divergent evolution is the differentiation of distinctly different species (or populations) from a common ancestral species (or population) (Figure 9.3.11). As time passes, natural selection or genetic drift may lead to the divergence of species or populations. Isolated species and populations accumulate genetic differences and their homologous features may become different, with different functions (for example, a human arm and a bat wing).

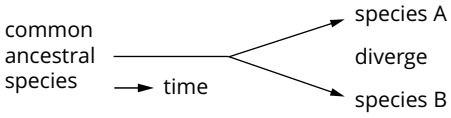


FIGURE 9.3.11 Divergent evolution.

Charles Darwin's finches are a classic example of divergent evolution. Darwin collected specimens of finches from the different Galapagos Islands (Figure 9.3.12). When he returned to England, the ornithologist John Gould examined the specimens and recognised them as related but as distinct species. Darwin had collected 13 different species of finch, with every island in the archipelago home to a number of species. Each species has a particular beak, body size and feeding behaviour that is advantageous for the conditions on the island on which they live. Over time, the generations within the population from the ancestral finches underwent adaptation due to environmental stresses and selective agents. This resulted in various phenotypes being more advantageous to the conditions of the islands, and the development of distinct species (Module 9.2).

FIGURE 9.3.12 Galapagos Islands, Ecuador.

Darwin's finches display the relationship between morphology and selective agents. In this case, different food types resulted in divergent evolution. This is an example of adaptive radiation (Figure 9.3.13). **Adaptive radiation** is the rapid divergent evolution of a large number of related species from a single common ancestor. The Galapagos finch genome sequence provides researchers with information about the process of divergent evolution (Case study 9.3.2).

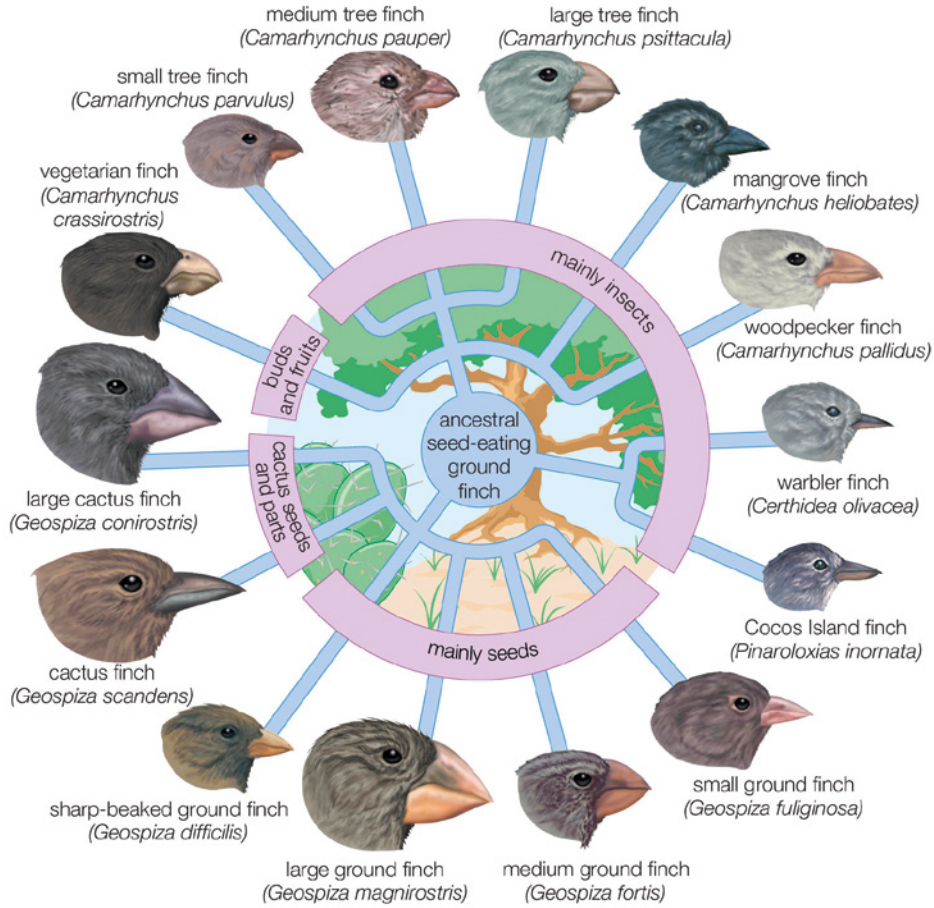


FIGURE 9.3.13 Adaptive radiation of Darwin's Galapagos finches. All these species have evolved from a common ancestor.

Australia has many unique marsupials. Marsupials are a subclass of mammal. They are thought to have evolved into the present range of familiar species through divergent evolution from placental mammals around 160–180 mya. It is proposed they migrated to the (current) North American continent and **Gondwana**, which then separated into the present South American and Australian landmasses.

Numerous allopatric, parapatric and sympatric speciation events have been hypothesised to influence the accumulation of genetic changes leading to divergent evolution and then macroevolution. Geological and topographical changes and occupation of new habitats are thought to have resulted in evolutionary radiation from 55 to 70 mya, with an array of different marsupial orders, genera and species arising. The family Macropodidae alone includes 10 genera and 65 species, including kangaroos, wallabies, wallaroos, quokkas, pademelons and tree kangaroos (Figure 9.3.14).

It is proposed that macropods diverged from a common marsupial ancestor approximately 53 mya, with modern kangaroos radiating from about 25 mya. For each genus today there are multiple species. Modern species have adapted to a browsing or grazing lifestyle, with teeth and digestive systems that are specialised for feeding on plant material. This example of adaptive radiation, like most, occurred through multiple divergences over millions of years.

Much of the speciation occurred after separation of the continents from the Gondwana land mass, so South America and Australia have different species of marsupial. Morphological evidence and comparative genomics provide the evidence of their pattern of divergence and radiation.

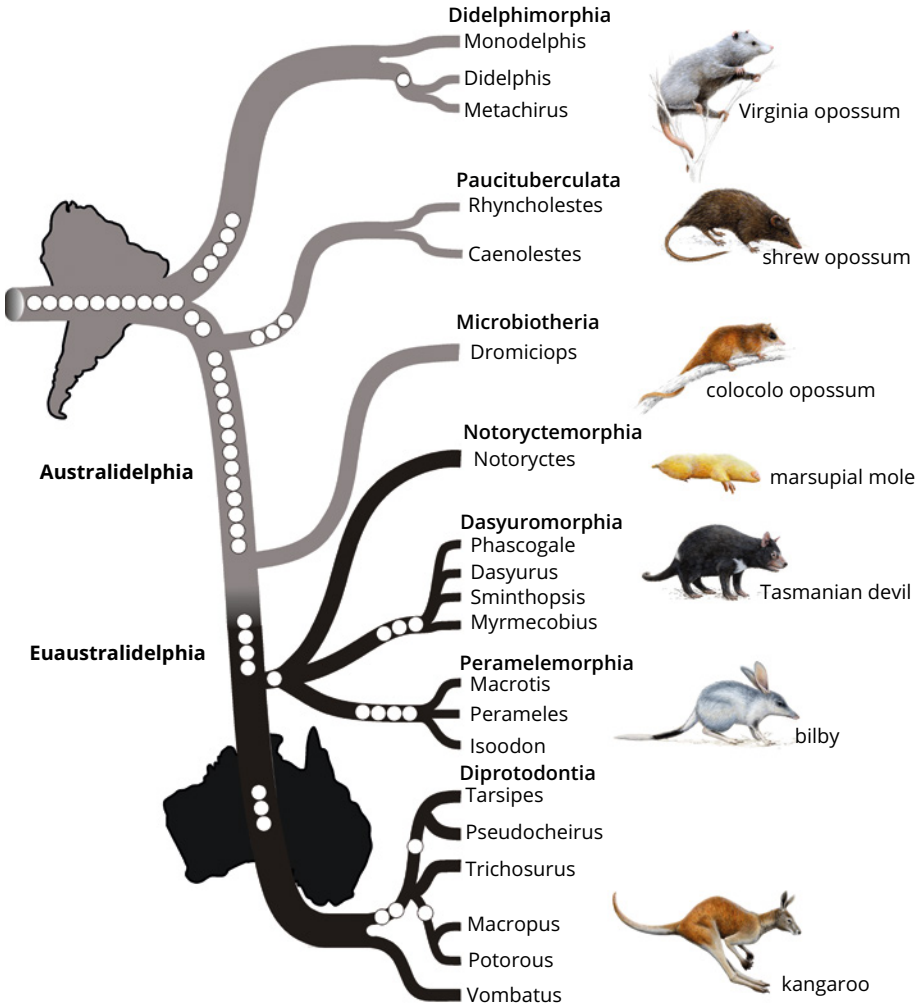


FIGURE 9.3.14 Evolutionary tree illustrating radiation of marsupials. This tree is based on the presence or absence of non-coding DNA elements (called retroposons), represented as the white spots. Retroposons insert into the genome and are inherited like other genes. Inheritance of different retroposons traces the radiation pattern of marsupials from South America to Australia.

WS 4.2.6

Convergent evolution

Convergent evolution is the evolution through natural selection of similar features in unrelated groups of organisms (Figure 9.3.15). Features with similar functions that have evolved from different ancestral structures are termed analogous. Unrelated species that have adapted to a particular environment in similar ways are said to have converged. They have evolved similarities in phenotype but remain distinct species that do not interbreed.

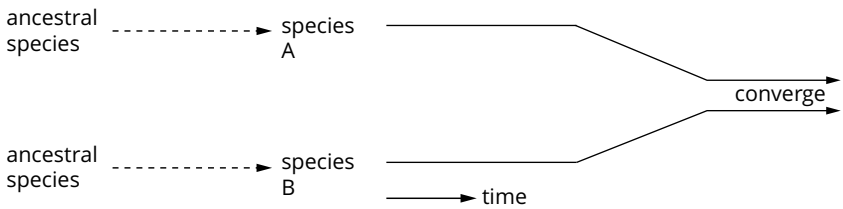


FIGURE 9.3.15 Convergent evolution.

The Australian marsupial sugar glider (*Petaurus breviceps*) and the American placental flying squirrel (*Glaucomys* sp.) have both developed large membranes between their fore and hind limbs that enable them to glide (Figure 9.3.16). This is an example of two unrelated species that have converged due to similar environments and lifestyles despite different origins.

FIGURE 9.3.16 (a) Australian marsupial sugar glider (*Petaurus breviceps*). (b) American flying squirrel (*Glaucomys* sp.).

FIGURE 9.3.17 Eye of a giant octopus.

The eyes of octopuses (Figure 9.3.17) and vertebrates are an example of convergent evolution. Even though octopuses and vertebrates have similar eye structures, the eyes have different origins.

The leaves of many Australian native shrubs in desert heathlands are hard, rigid, small and adapted to dry conditions. They resemble one another even though they are unrelated (for example, peas, banksias and acacias).

The thylacine, also known as the Tasmanian tiger (*Thylacinus cynocephalus*) (Figure 9.3.18), is also a good example of convergent evolution. The heads of thylacines are very similar in shape to those of individuals in the Canid family (dogs), yet they are not closely related. Thylacines are marsupials and dogs are placental mammals. They share a common ancestor around 160 mya. Genomic analysis points to similar genetic variants in regulatory genes that control structural development as being the main genetic factors behind the similar morphology. One or a few mutations in a regulatory gene can affect several structural genes and have a large impact on phenotype.

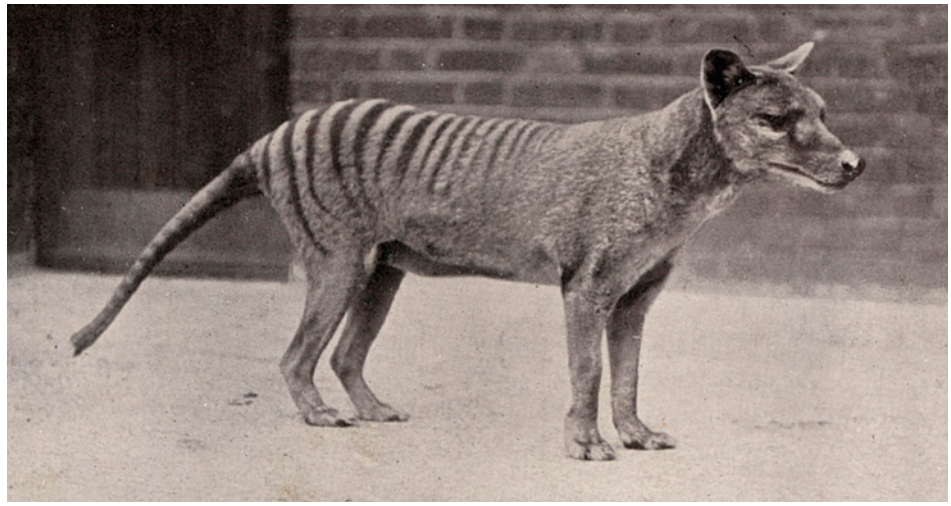


FIGURE 9.3.18 Thylacine at London Zoo around 1910

WS 4.2.5 PA 4.2.1 PA 4.2.2

Parallel evolution

Parallel evolution occurs when related species evolve similar features independently (Figure 9.3.19). In some cases, different mutations lead to a similar phenotypic change in the parallel lineages. In other cases, the same mutation occurs in each lineage.

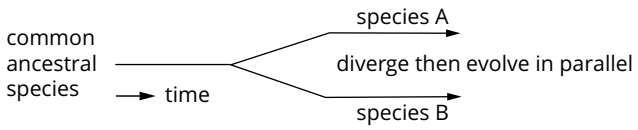


FIGURE 9.3.19 Parallel evolution.

For example, within the eucalypts, a number of species have evolved a white, waxy coating on their leaves that protects them from frost damage at high altitudes. Snow gum (*Eucalyptus pauciflora*) and argyle apple (*Eucalyptus cinerea*) both have waxy leaves, a trait that evolved independently in each lineage after divergence from the common ancestor. Another example is the white eye mutation that is common among several related species of *Drosophila* flies. The same mutation occurs independently in the species and thus the white eye trait has evolved, in parallel, more than once.

Polydactyly is a common inherited trait in chickens (Figure 9.3.20). Chickens normally have four toes but polydactyly causes five or six toes to develop on one or both feet. Genetic evidence indicates the trait has evolved in parallel in some Chinese and European chicken breeds. A regulatory region with two alleles, *A* and *C*, corresponds to the trait in some Chinese breeds. The Chinese breeds with normal four-toed feet are genotype *CC*, while those with polydactyly have the *AC* or *AA* genotype. However, the European breeds do not share the genotype. French Houdan chickens with or without polydactyly have the *CC* genotype, so other factors must contribute to the polydactyly, while the *AC* locus is not linked to polydactyly in Italian white leghorn chickens at all (Table 9.3.6). A genome-wide scan identified different genes associated with polydactyly in the European breeds, showing that the same trait arose from different mutations and the different breeds have evolved in parallel.

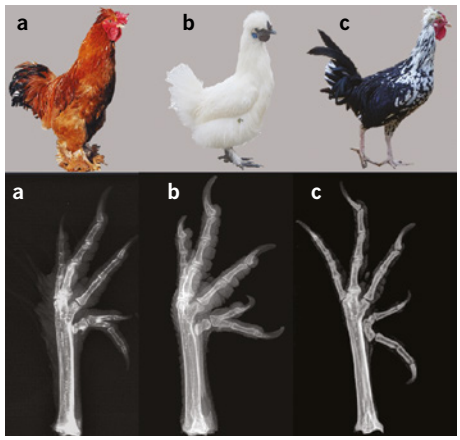


FIGURE 9.3.20 Polydactyly in chickens. Five toes instead of the normal four shown in X-rays of the foot bones from (a) Beijing-You chicken, (b) Silkie chicken and (c) Houdan chicken.

TABLE 9.3.6 Genotypes of five chicken breeds in birds with and without polydactyly

Breed name	Number							Origin
	Total	Polydactyly			Non-polydactyly			
		CC	*AC*	*AA*	*CC*	*AC*	*AA*	
Beijing-You	236	0	51	4	181	0	0	China
Silkie	110	0	39	62	9	0	0	China
Jiningbairi	38	0	18	0	20	0	0	China
Houdan	86	0	43	0	43	0	0	France
White leghorn	96	0	0	0	96	0	0	Italy

Coevolution

Species that interact closely exert selection pressures on each other. Both species also experience similar environmental conditions. In such situations, **coevolution** occurs, with the two species evolving together in a reciprocal response to various selection pressures.

New variations of flowers appear through mutation, and these may be more likely to survive and produce seeds. As a result, some pollinators will be more suited to these flowers and will evolve alongside the flowers. In the case of *Lonicera gracilipes*, an early spring flower (Figure 9.3.21a), the long, narrow corolla tube of the flower fits the long tongue of the andrenid bee species *Andrena lonicerae* and prevents pollinators with a short tongue from reaching the pollen. A closely related bee, *A. halictoides*, has an intermediate phenotype, while the distantly related *A. hebes* has short mouthparts (Figure 9.3.21b).

Molecular phylogeny shows that *A. lonicerae* and *A. halictoides* are a monophyletic sister group and that *A. lonicerae is derived* from the ancestral *A. halictoides* (Figure 9.3.21c). That is, this bee taxa has evolved structural and behavioural adaptations finely tuned to feeding on this one flower species, and the flower has evolved a structure that relies on this bee for pollination.

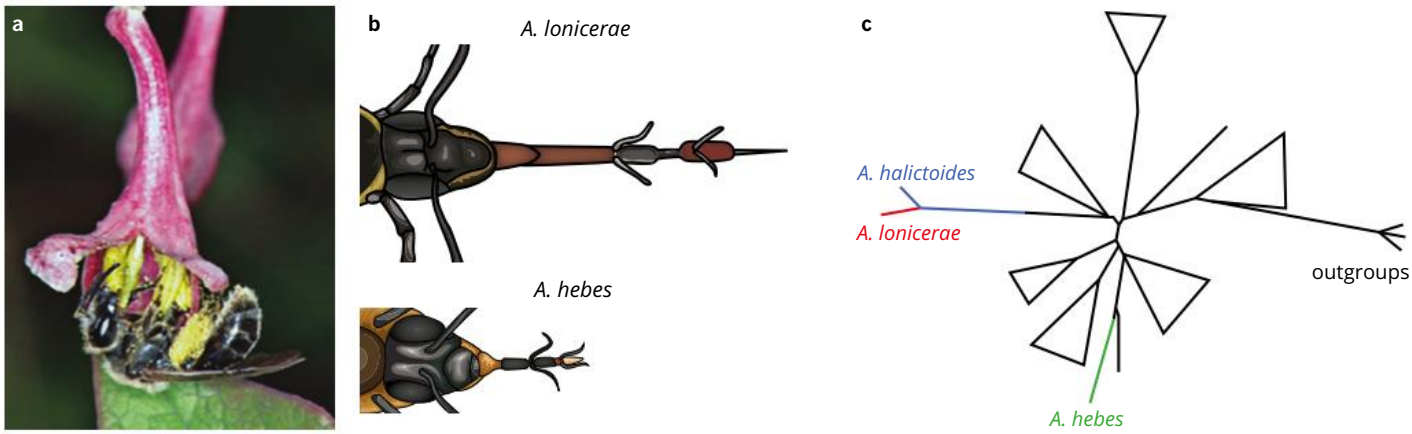


FIGURE 9.3.21 (a) *Lonicera gracilipes* produces nectar collected almost exclusively by andrenid bees. (b) Head and mouthparts of the bee *A. lonicerae*, the almost exclusive pollinator of this flower, compared to a distantly related bee, *A. hebes*. (c) Phylogeny of *Andrena* bees showing the *A. lonicerae* clade (red), the *A. halictoides* clade (blue) and the *A. hebes* clade (green).

Coevolution can also be observed in predator–prey relationships. When predators pick off the weaker prey, stronger individuals are left to reproduce. The next generation of predators will need to be stronger and faster to keep up with the strengthening features of the prey.

GENETIC DIVERSITY: SPECIES SURVIVAL OR EXTINCTION

Genetic diversity refers to all of the genetically determined characteristics of a species. A high level of genetic diversity allows populations to survive new or changing environments, which may lead to adaptation. Conversely, low genetic diversity increases the risk of extinction, as seen when species or populations go through a genetic bottleneck.

Bottleneck effect

The number of individuals in a population can be rapidly reduced because of a random event, often a natural disaster. Human activities, such as hunting and land clearance have also greatly reduced the number of individuals in wild populations of plants and animals. The phenotype of an individual is unlikely to significantly increase its chances of surviving a natural disaster, such as a volcanic eruption, tidal wave or landslide. The individuals that survive will do so by chance. The allele frequencies of the remaining population are unlikely to reflect those of the original population and genetic drift (Module 9.2) can occur.

The bottleneck effect describes the impact on the remaining population (Figure 9.3.22). Because of the reduced population size, the possible reproductive pairings are limited, which leads to high levels of inbreeding. Inbreeding results in reduced variation in the population and an increase in the numbers of homozygous individuals. The smaller the population, the greater the bottleneck effect, an impact of genetic drift. Alleles may be lost from the gene pool immediately after the natural disaster or be bred out in only a few generations. The lowered variation may make the population more vulnerable to environmental change.

FIGURE 9.3.22 The mountain pygmy possum has a declining population and reduced genetic diversity. This population bottleneck is a result of bushfires, and habitat loss from land clearance and development around Mount Buller, Victoria.

Low genetic diversity in Tasmanian carnivores

Fossils and ancient indigenous artworks in northern Australia indicate that the thylacine once inhabited most of the country. During the course of human habitation in Australia, its range decreased until it was confined to Tasmania. It faced competition from the introduced dingo and was close to being extinct in the wild, when people hunted it for a bounty. The last individual died at Hobart Zoo in 1936.

It is inferred from the complete genome sequence of a preserved thylacine fetus that population numbers and genetic diversity started decreasing 70 000 years ago or earlier, before human habitation of Australia 50–60 000 years ago. The cause may have been a cooling climate and the consequent habitat change. Low genetic diversity leaves a population less able to adapt to changing environments, including the effects of human habitation, such as hunting and habitat loss, putting the species at greater risk of extinction.

The genome of another Tasmanian marsupial, the Tasmanian devil, shows that it also has very low genetic diversity, which most likely increases its susceptibility to the facial tumour disease that is currently threatening their survival as a species and susceptibility to other extinction risk factors such as **habitat fragmentation**.

CASE STUDY 9.3.2

Phylogenomics for Darwin's finches

In 2015, scientists sequenced the full genome of 120 different individual finches representing each of the groups of Galapagos finch (Figure 9.3.23), as well as related finches from the Cocos Islands. In the figures below, the letter after the species name represents the island where the bird lives. *G. difficilis*_F comes from Fernandina Island and *G. difficilis*_P lives on Pinta Island. Two related tanager species, *Tiaris bicolor* and *Loxigilla noctis*, were also sequenced as outgroups.

The procedures involved in large genomic phylogeny projects include:

- extracting the DNA
- preparing a library of short, overlapping DNA fragments (400 bp), which are more manageable to sequence than longer molecules
- performing DNA sequencing of all chromosomes and the mitochondrial DNA. The nuclear genome size was more than 1 Gb (1 000 000 000 bp) and contained more than 16 000 protein coding genes. The mitochondrial genome of birds is around 17 000 bp with 37 genes
- performing multiple sequence alignments
- computation to identify regulatory sequences, coding sequences and non-coding regions
- calculating the number of base changes for statistical analysis of divergence and genetic distance
- constructing phylogenetic trees.

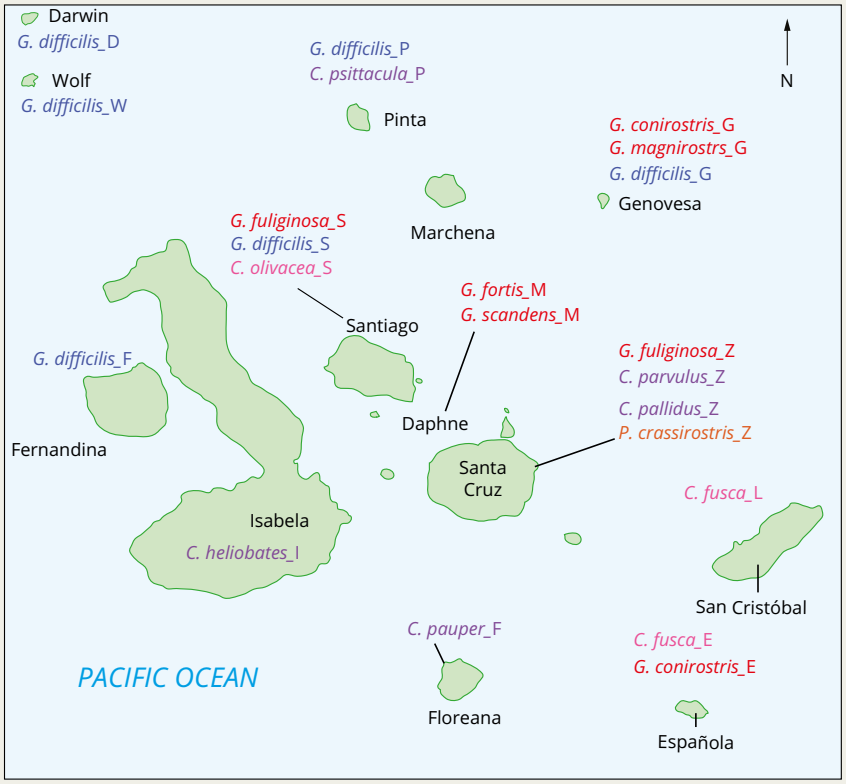


FIGURE 9.3.23 The Galapagos Islands and location of birds sampled for whole genome sequencing. Different species are indicated by colour.

Statistical analyses of the data, using previously determined mutation rates, estimates the degrees of relatedness and genetic distance between individuals to infer divergence times between taxa

The phylogenetic tree based on diversity in autosomal DNA, shown in Figure 9.3.24, provides an estimate of a major divergence point between the warbler finches and the other genera that occurred on the Galapagos Islands around 900 000 years ago, and estimates of subsequent divergences between species. Adaptive radiations occurred more recently (indicated by the groups of small branches).

This whole genome sequence shows differences from phylogenies based on morphology and on previous analyses of mitochondrial DNA, individual gene sequences or microsatellite DNA (non-coding DNA sequences). The taxa that show variation from previous phylogenies are marked with an asterisk (*). For example, two separate populations of the cactus finch *G. conirostris* (red text), from Española (E) and Genovesa (G) islands, show enough variation in their whole genome sequences to place them in separate taxa. The place of the sharp-beaked ground finch, *G. difficilis*, is also more distant on this tree than a tree based on morphology and behaviour. Compare the tree in Figure 9.3.24 to Figure 2.2.19 on page 39.

Beak morphology in Galapagos finches has been studied extensively. Adaptations in beak morphology on a single island occur in response to environmental change such as drought. The genomic analysis has identified the *ALX1* gene with two alleles that are linked to beak morphology: *P* (pointy) and *B* allele (blunt). In Module 9.2, you saw how the allele frequency at this locus corresponds to the beak size of *G. fortis* in response to drought.

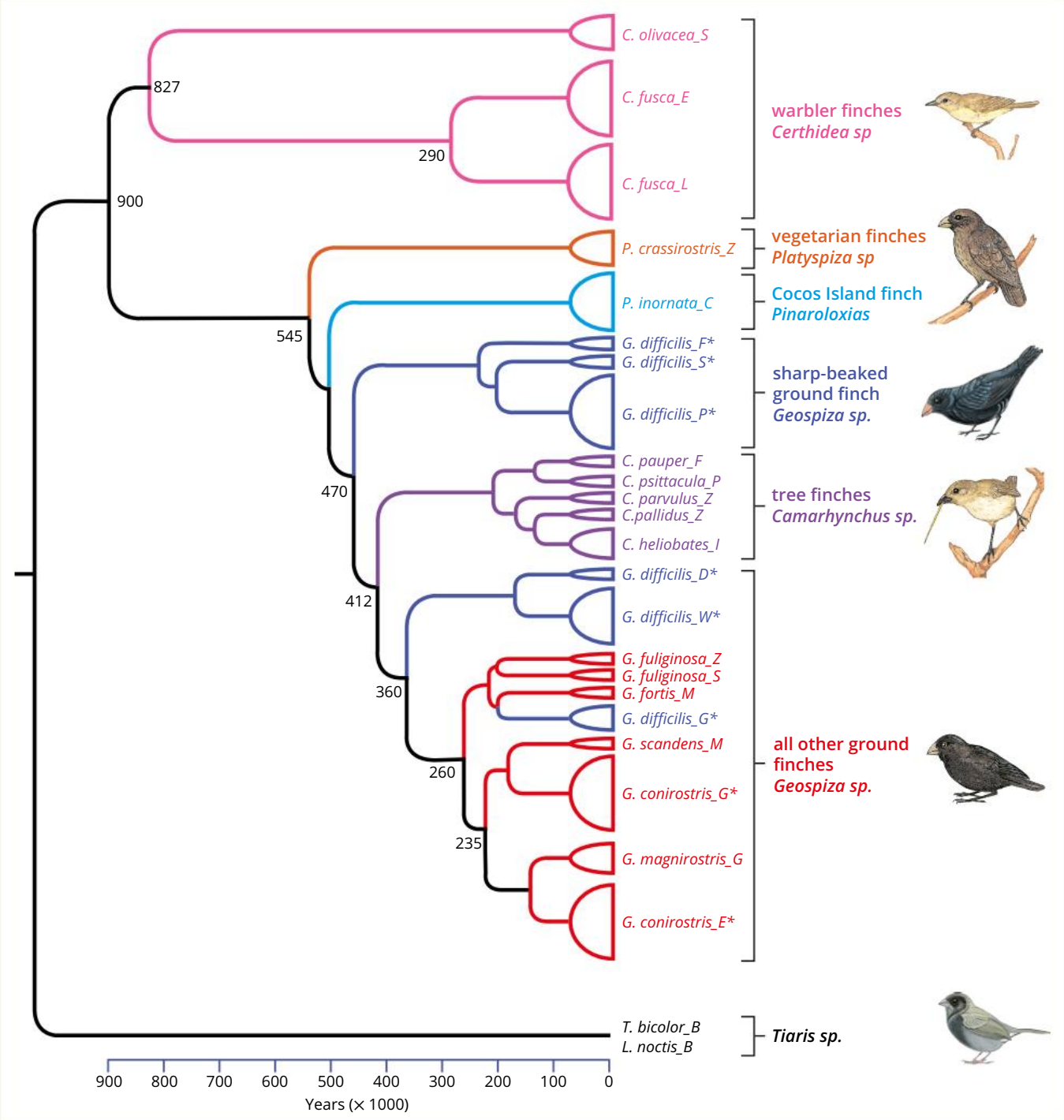


FIGURE 9.3.24 Divergent evolution in Galapagos finches. Phylogenetic tree based on genome diversity analysis in autosomal DNA. Groups showing variation from morphological taxonomy are marked (*). An estimated timescale of 900 000 years is shown. Suggested times for major divergences are given. A cone shape at the end of a branch represents a group of small branches.

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CASE STUDY 9.3.2

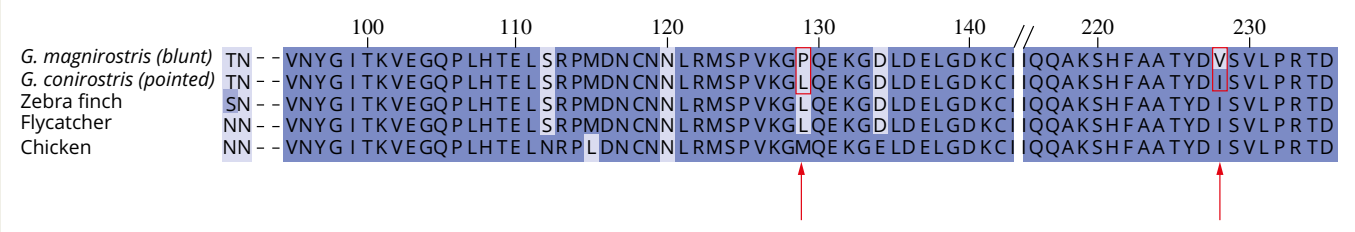


FIGURE 9.3.25 Two sections of ALX1 amino acid sequence with amino acid changes marked by red boxes. The amino acid differences correlate with pointy and blunt-beak phenotypes.

ALX1 is a transcription factor that acts in early vertebrate development and is important for the normal development of structures in the head, including mouth, eyes, nose and beak. Variations in regulatory genes acting in development are considered to be important in morphological adaptations. The researchers identified the base changes and amino acid differences between the *ALX1* alleles.

The divergence in the *P* and *B* variants of *ALX1* coincides with the major divergence between warbler finches and other finches. All of the warbler finches have a pointy-beak phenotype and are homozygous *PP*, while genotypes *BB* and *BP* (blunt-beak phenotype) are present in the other genera. The ALX1 protein has some amino acid differences between *Geospiza magnirostris* (homozygous *BB*) and *G. conirostris*, which correspond to blunt and pointed beaks (Figure 9.3.25).

The researchers also found evidence of gene flow, and concluded that several of the species interbreed, leading to hybrids. In 2017, the same group of researchers reported that a new species evolved from a hybridisation after a large cactus finch, *G. conirostris*, migrated to the Daphne Major island, mated with a resident medium ground finch, *G. fortis*, and produced viable hybrid offspring, which have now produced a new lineage in only a few generations.

Review

1. Identify the evidence gathered for multiple sequence alignment of genome sequences and describe the objective of the method.
2. Argue why the full nuclear and mitochondrial genome sequence of finches is more informative than the mitochondrial sequence alone.
3. State the estimated time range when the Cocos finches diverged from the Galapagos Island finches.
4. From Figure 9.3.24 (page 493), identify the time that includes all the adaptive radiations of finches, excluding the warbler and vegetarian finches.
5. Compare the amino acid sequences of ALX1 between Darwin's finches and the other birds to confirm whether or not the sequence is highly conserved.
6. *Geospiza fortis* populations on Daphne Major are often heterozygous *BP* at the beak shape *ALX1* locus. If some neighbouring homozygous *BB* (blunt-beaked) *G. fortis* adults took up residence in a heterozygous *BP* population and mated:
   - **a** describe the effect on the frequency of the *B* and *P* alleles
   - **b** name the process occurring in this situation.
7. Explain whether the present-day finches are a result of convergent, divergent or parallel evolution from the common ancestor, which is assumed to have arrived in the Galapagos Islands around 2.3 mya from South America.

9.3 Review

SUMMARY

- Macroevolution is the variation of allele frequencies at or above the level of species over geological time, resulting in the divergence of taxonomic groups and new taxonomic groups
- A species is a group of individuals that can produce viable, fertile offspring through interbreeding and has a gene pool that is isolated from the gene pools of other species.
- Populations may evolve into different species as a result of being fragmented through various genetic isolating mechanisms.
- Isolating mechanisms can be prezygotic, preventing fertilisation of gametes of different species, or postzygotic, preventing production of viable offspring after fertilisation.
- Speciation is the evolution of new species from an ancestral species. The new species can no longer produce viable offspring if they interbreed.
- Speciation involves genetic isolation between diverging populations or a single species gradually changing over time.
- Different types of speciation involve different genetic isolating mechanisms.
  - Allopatric speciation occurs when a population is divided by a geographical barrier.
  - Sympatric speciation occurs when populations develop behavioural, morphological or another isolation mode that leads to reproductive separation.
  - Parapatric speciation occurs when populations are not geographically isolated but there is variation in habitat conditions that separates reproductive groups.
- When different species originate from a common ancestor, they continue to diversify. There are four different patterns of diversification.
  - Divergent evolution is the differentiation of distinctly different species from a common ancestor, in which the shared features can adopt different functions. Adaptive radiation is a form of divergent evolution in which a common ancestor gives rise to a number of new species rapidly, particularly when a change in the environment opens new environmental niches.
  - Convergent evolution is the development of similar features in unrelated species through natural selection.
  - Parallel evolution occurs when related species evolve similar features independently.
  - Coevolution occurs when two interacting species evolve together in a reciprocal response to selection pressures.
- When a population is suddenly reduced in size (for example after a natural disaster), the resulting loss of genetic diversity can lead to a bottleneck effect, which increases the risk of extinction.
- Data about gene flow and allele frequency from different populations can be used to determine speciation.

KEY QUESTIONS

Retrieval

1 Define speciation.

2 Describe how microevolutionary change relates to macroevolution.

3 Other than genetic isolation, name two other factors that contribute to speciation.

4 Name the type of prezygotic isolating mechanisms that prevent gene flow between populations during allopatric speciation. Give an example.

5 Identify some geographical factors that can result in allopatric speciation.

Comprehension

6 Copy and complete the following table, summarising different patterns of evolution.

Patterns of evolution

Pattern of evolution	Definition	Arrow diagram showing change over time	Example
divergent			
convergent			
parallel			

continued over page

9.3 Review *continued*

7 Explain the importance of genetic isolation in speciation.

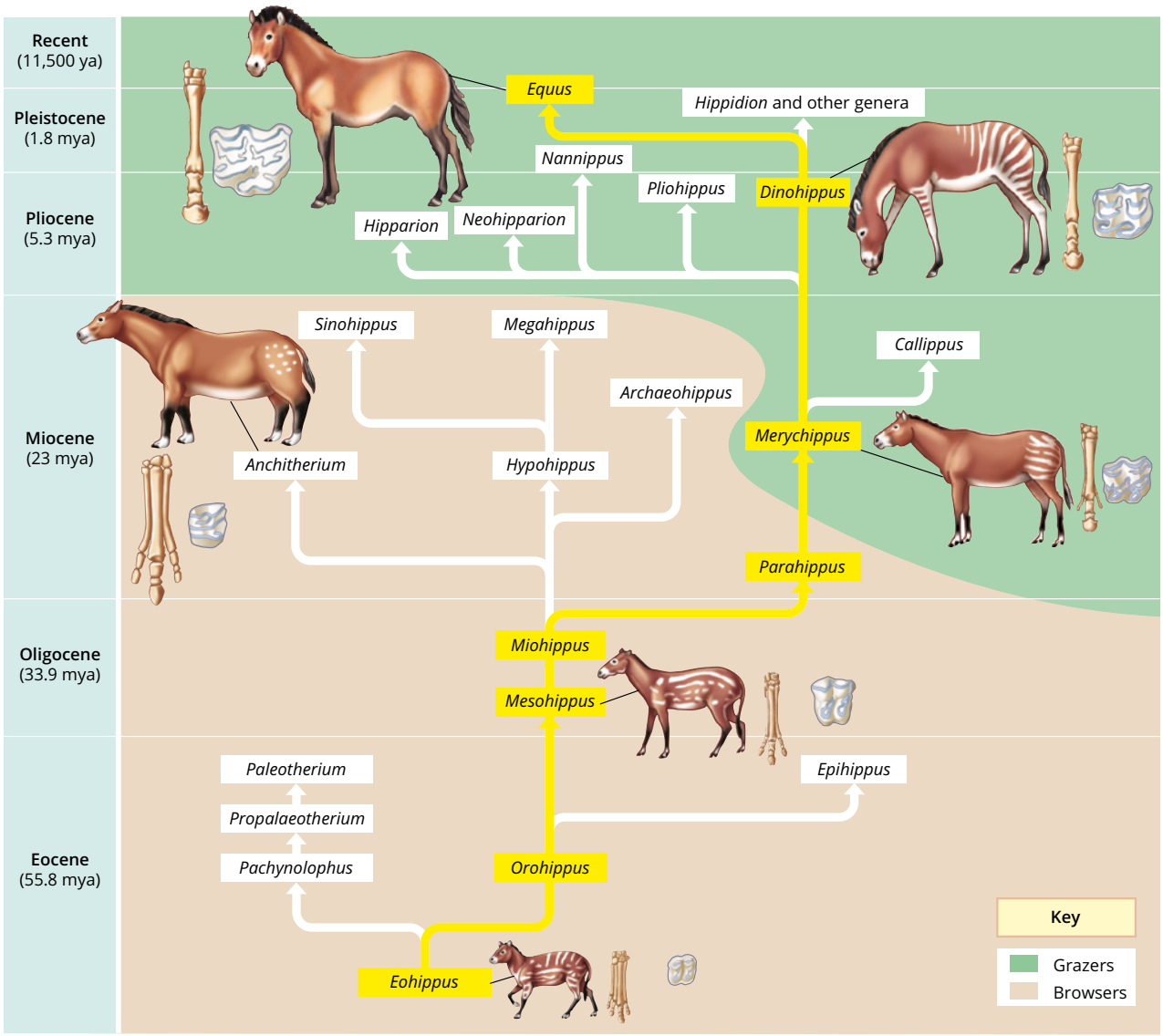
8 Australia's southern boobook owl is closely related to the New Zealand morepork owl. Numerous microevolutionary changes have resulted in two species that are separated by a geographical barrier. Discuss the assumption that could be reached about the offspring from an attempt to crossbreed a morepork owl with a southern boobook owl.

9 There is striking similarity between the eye of a vertebrate, such as a shark, and that of an octopus.
   a Name the type of evolution involved.
   b Argue a plausible reason for the evolution of a similar type of eye in octopuses and vertebrates.

10 Explain the notion that low genetic diversity in Tasmanian devils relates to an increased risk of extinction due to factors such as devil facial tumour disease.

Analysis

11 View the diagram below, which represents an interpretation of evidence for horse evolution.
   a Identify the most recent proposed common ancestor of the modern horse, *Equus*, and *Merychippus*.
   b Identify what behaviour may have provided a selection advantage between *Parahippus* and *Merychippus*.

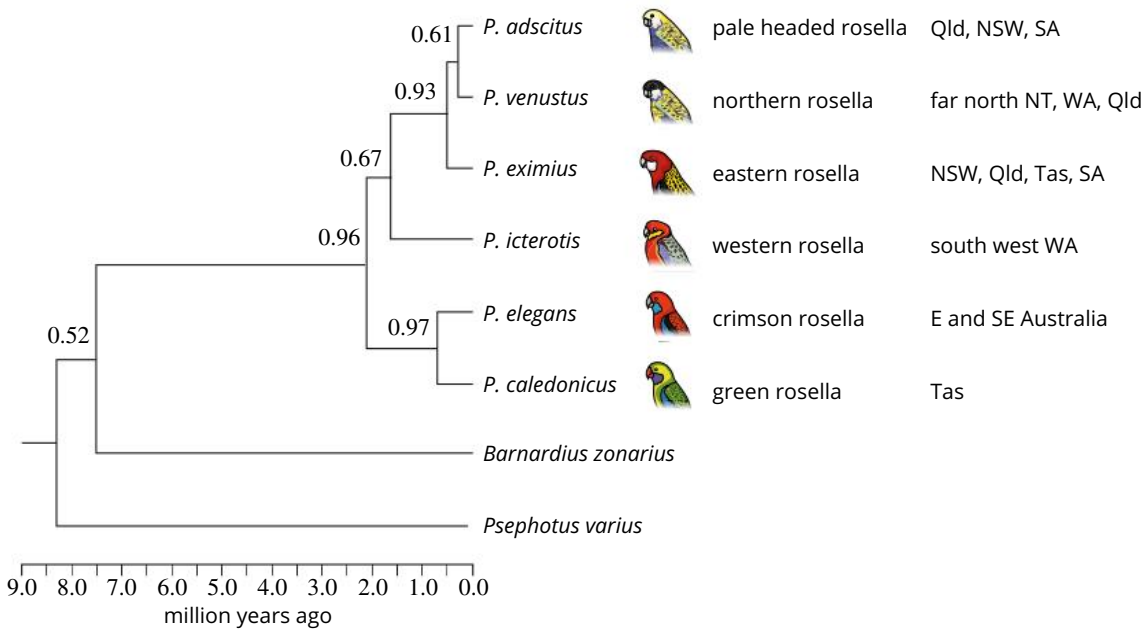


12 View the diagram below of a proposed phylogenetic tree of the six species of Australian rosella. Some of the species have overlapping ranges. Interbreeding and hybridisation can occur.

a Deduce a genetically determined factor that may have acted as a selecting agent during speciation of *P. venustus* and *P. eximius*.

b Propose a mechanism contributing to the speciation of *P. icterotis*.

13 Evaluate the influences of geographical separation and gene flow in the speciation of *Drosophila* flies inhabiting the north and south walls of Evolution Canyon. Use the genotype data from the *hsp70Ba* locus in Table 9.3.5 (page 484) and your understanding of the principles of speciation.



Modelling evolution and measuring population genetics

Research and planning

Aim

- To calculate phenotypic and allelic frequencies
- To apply the Hardy–Weinberg theorem to a range of modelled populations
- To examine the effect of natural selection on allelic frequencies over time
- To identify the conditions under which the Hardy–Weinberg theorem applies

Rationale (scientific background to the experiment)

In the early 1900s, mathematicians G. Hardy and W. Weinberg used their knowledge of binomial expansion and applied it to population genetics (microevolution). Microevolution involves the change in allele frequency over time. A population that is in Hardy–Weinberg equilibrium shows no change in allele frequency over time; it is a non-evolving population. In order for a population to comply with the Hardy–Weinberg equilibrium, five conditions must be met:

- The population is infinitely large. This reduces the probability that genetic drift (random change in allele frequency) will occur.
- Mating is random. No sexual selection.
- No natural selection. All genotypes have the same chance of survival.
- No mutation of alleles. No new alleles enter the population.
- No gene flow. No emigration or immigration.

A Hardy–Weinberg population cannot exist in the real world, as it is impossible to meet all five of the conditions simultaneously. The Hardy–Weinberg principle simply provides a baseline to determine whether or not allele frequencies have changed in a population and, thus, whether evolution has occurred. There are two equations which are used when working with the Hardy–Weinberg theorem:

$p + q = 1$

where

p = the frequency of the dominant allele in the population

q = the frequency of the recessive allele in the population

$p^2 + 2pq + q^2 = 1$

where

p^2 = the frequency of the homozygous dominant genotype in a population

$2pq$ = the frequency of the heterozygous genotype in a population

q^2 = the frequency of the homozygous recessive genotype in a population

Timing

60 minutes

Materials

- calculator with square root function
- 4 index cards (2 labelled ‘a’, 2 labelled ‘A’)
- coin

Method

Risk assessment

Assessment of risks includes chemical hazards and physical hazards. Before you commence this practical activity, you must conduct a risk assessment. Complete the template in your *Pearson Biology 12 Queensland Skills and Assessment* book or download it from your eBook.

Case I: Testing an ideal Hardy–Weinberg population

The entire class will be used to simulate an ideal breeding population. You will need sufficient room to move around. In this simulation, gender and genotype are irrelevant to mate selection. The class will simulate a population of randomly mating heterozygous individuals with an initial frequency of 0.5 for the dominant allele *A* and 0.5 for the recessive allele *a*. The initial genotype frequencies will be 0.25 *AA*, 0.5 *Aa*, and 0.25 *aa* (half of the class will be *Aa*, one-quarter will be *AA* and one-quarter will be *aa*). This represents the genotypic frequencies in an ideal population according to the Hardy–Weinberg model.

1. Choose a student at random as your partner to simulate random pairing.
2. Decide between you and your partner which one of you will be number 1 and number 2.
3. Turn your 4 cards face down so that the letters do not show and shuffle the cards.
4. Pick up the top card and look at the letter. This is the allele that you contribute to the next generation.
5. Have your partner now go through the same process.
6. Put the 2 cards together, and you have the 2 alleles of the first ‘offspring’ of the students.
7. Student 1 records this as their initial genotype in the Case I portion of the Analysis section.
8. Student 2 needs to produce an offspring. (You and your partner need to produce a total of 2 offspring.) All 4 cards must be reshuffled and the process repeated to produce a second offspring.

9 Student 2 records this as their initial genotype in the Case I portion of the Analysis section.

10 This marks the end of the short reproductive career of the first generation. You and your partner now should assume the genotype of each of your offspring. Student 1 assumes the genotype of the first offspring and student 2 assumes the genotype of the second offspring.

11 Randomly find another partner to 'mate' with to produce offspring for the next generation. Remember, the sex and genotype of your partner is irrelevant. Follow the same procedure as you did for the first generation to produce a total of five generations, being sure to record your new genotype after each mating event.

12 Collect class data for all 5 generations and record this in Table 1 in the Analysis section.

Case II: Selection

In this case, you will modify the simulation followed in Case I to make it more realistic. In the natural environment, not all genotypes have the same rate of survival. In nature, some genotypes confer an advantage over others, which are selected against.

Infantile Tay Sach's disease is an example of a human disorder that is selected against in nature. This condition is caused by a mutation on the allele that codes for a lysosomal enzyme that breaks down fatty acid derivatives. When this enzyme is non-functional, lipids aggregate in the brain of affected individuals, eventually causing death. Individuals who are homozygous recessive do not survive past the age of five and do not reach reproductive maturity.

For this simulation, you will assume that the homozygous recessive individuals are selected against and do not survive. It will also be assumed that the heterozygous and homozygous dominant individuals survive 100% of the time.

13 Follow the procedure for Case I to produce offspring through 5 generations with your initial genotype as *Aa*. This time there is one important difference. Whenever your offspring receives two recessive alleles, *aa*, it does not survive (and does not reproduce). If you produce an offspring that is *aa*, you and your partner must keep trying to reproduce until you produce a viable offspring (to maintain the same population size). Remember that the homozygous recessive genotype is selected against all of the time.

14 Repeat the process until your partner also produces and records a viable offspring.

15 Collect class data for all 5 generations and record this in Table 2 in the Analysis section.

Case III: Heterozygote advantage

In this case, you will investigate the effect that heterozygote advantage has on allele frequencies. In some populations, individuals who are heterozygous have a selective advantage over the individuals who have other genotypes. This is exactly the case with the sickle-cell anaemia allele in certain populations. Sickle-cell anaemia is caused by a mutation on a single allele. Homozygous recessive individuals do not produce normal haemoglobin proteins. These proteins can crystallise in the red blood cells, causing them to form a sickle shape. Individuals with this disorder are chronically anaemic and experience periodic episodes of pain. Many of these people do not survive to reach reproductive maturity. One would expect that the recessive allele would have a relatively low frequency in all populations. However, certain populations show an unexpectedly high frequency of the recessive allele because heterozygous individuals are slightly more resistant to malaria when compared to homozygous individuals.

16 Follow the procedure for Case II, with your initial genotype as *Aa*. Record your initial genotype in the Case III portion of the Analysis section. This time, when you produce an offspring with the *AA* genotype, you will flip a coin. If the coin lands on heads, the *AA* offspring survives. If it lands on tails, the *AA* offspring dies before reproductive maturity. Simulate 5 generations. Remember that whenever your offspring receives 2 recessive alleles, *aa*, it does not survive, and that the homozygous dominant offspring (*AA*) will only survive if the coin lands on heads. If you produce an *aa* offspring or if your *AA* offspring dies as a result of the coin flip, you and your partner must keep trying to reproduce until you produce a viable offspring (to maintain the same population size.)

17 Repeat the process until your partner also produces and records a viable offspring.

18 Collect class data for all 5 generations and record this in Table 3 in the Analysis section.

Analysing

Raw data

Case I: Testing an ideal Hardy–Weinberg population

My initial genotype: _____.

Generation 1 genotype: _____.

Generation 2 genotype: _____.

Generation 3 genotype: _____.

Generation 4 genotype: _____.

Generation 5 genotype:_____.

1 Gather class data. Count the number of offspring in each generation with the following genotypes: *AA*, *Aa* and aa. Record in Table 1.

2 Simplify genotypic ratios by reducing to lowest terms.

TABLE 1 Case I: Testing an ideal Hardy-Weinberg population

Generation	Class totals for each genotype		
	AA	***Aa***	***aa***
1			
2			
3			
4			
5			

Case II: Selection

My initial genotype: _____.

Generation 1 genotype: _____.

Generation 2 genotype: _____.

Generation 3 genotype: _____.

Generation 4 genotype: _____.

Generation 5 genotype:_____.

3 Gather class data. Count the number of offspring in each generation with the following genotypes: *AA*, *Aa* and *aa*. Record in Table 2.

4 Simplify genotypic ratios by reducing to lowest terms.

TABLE 2 Case II – Selection

Generation	Class totals for each genotype		
	AA	***Aa***	***aa***
F_1			
F_2			
F_3			
F_4			
F_5			

Case III: Heterozygous advantage

My initial genotype: _____.

Generation 1 genotype: _____.

Generation 2 genotype: _____.

Generation 3 genotype: _____.

Generation 4 genotype: _____.

Generation 5 genotype:_____.

5 Gather class data. Count the number of offspring in each generation with the following genotypes: *AA*, *Aa* and *aa*. Record in Table 3.

6 Simplify genotypic ratios by reducing to lowest terms.

TABLE 3 Case III – Heterozygous advantage

Generation	Class totals for each genotype		
	AA	***Aa***	***aa***
1			
2			
3			
4			

Processed data

7 Use the genotypic ratios above to determine the phenotypic ratios for F_1 and F_5. Record the data in Table 4.

TABLE 4 Phenotypic ratios

Case	Phenotypic ratio			
	F_1		F_5	
	Dominant	**Recessive**	**Dominant**	**Recessive**
Case I: Ideal population				
Case II: Selection				
Case III: Heterozygous advantage				

8 Calculate the allele frequencies for F_1 to F_5 using the formulas below. Record the data in Table 5.

Total number of alleles in the population = $n \times 2$, where n = population size

Total *A* alleles = 2 × number of offspring with genotype *AA* + 1 × number of offspring with genotype *Aa*

p = Total number of *A* alleles ÷ total number of alleles in the population

Total *a* alleles = 2 × number of offspring with genotype *aa* + 1 × number of offspring with genotype *Aa*

q = Total number of *a* alleles ÷ total number of alleles in the population

TABLE 5 Allele frequencies

Case	Allele frequency											
	F_1		F_1		F_2		F_3		F_4		F_5	
	p	*q*	*p*	*q*	*p*	*q*	*p*	*q*	*p*	*q*	*p*	*q*
Case I: Ideal population												
Case II: Selection												
Case III: Heterozygous advantage												

9 In a single graph, chart the change in the recessive allele frequency, *q*, over time for each of the scenarios.

➤ Reflect and check that your data analysis demonstrates these characteristics

- ☐ Effective investigation of phenomena is demonstrated by the collection of sufficient and relevant raw data.
- ☐ Accurate application of algorithms, visual and graphical representations of data is demonstrated by appropriate processing and presentation of data to aid the analysis and interpretation of data.

Analysis

10 State what the Hardy–Weinberg equation predicts for the values of *p* and *q* in the ideal population.

11 What assumption(s) of the Hardy–Weinberg equilibrium were not followed in the simulation in:

a Case I

b Case II

c Case II

12 Compare the phenotypic ratios of F_1 and F_5 in each of the above simulated cases. Explain any differences seen.

13 Compare the allele frequencies of F_1 and F_5 in each of the above simulated cases. Explain any differences seen.

14 Referring to the graph you drew, explain how natural selection affects the allele frequencies of a population over time.

15 Explain whether a recessive allele that causes disease could be completely removed from the populations of Case II or Case III.

➤ Reflect and check that your analysis demonstrates these characteristics

- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough and appropriate error analysis.
- ☐ Systematic and effective analysis of evidence is demonstrated by a thorough identification of relevant trends, patterns and relationships.
- ☐ Insightful and valid interpretation of evidence is demonstrated by drawing a valid and defensible conclusion based on the analysis.

Interpreting and communicating

Conclusion

1 Summarise the conditions under which the Hardy-Weinberg principle applies and how it can be applied.

Improvements

2 If you were to repeat this experiment, identify the steps that you would do differently. Consider how you:

a might change the methodology

b might improve your technique

c could reduce error and uncertainty

Extension

3 Explain the importance of a large population size in maintaining genetic diversity.

4 Explain whether it is possible to eradicate a lethal recessive allele in a very large population.

5 Explain the significance of the heterozygous condition in maintaining genetic variation in a population.

➤ Reflect and check that your evaluation demonstrates these characteristics

- ☐ Critical evaluation of processes is demonstrated by a discussion of the reliability and validity of the experimental process supported by evidence such as the quality of the data (as quantified in the error analysis).
- ☐ Critical evaluation of the conclusion is demonstrated by a discussion of the veracity of the conclusions with respect to the error analysis and limitations or sufficiency of the data.
- ☐ Insightful evaluation of processes and conclusions is demonstrated by a suggestion of improvements or extensions to the experiment that are logically derived from the analysis of the evidence.

Chapter review

WS 4.2.7 WS 4.2.8

09

KEY TERMS

adaptive radiation
allele frequency
allopatric speciation
artificial selection
background extinction
biological fitness
bottleneck effect
coevolution
common ancestor
comparative genomics
convergent evolution
Darwinian theory
directional selection
disruptive selection
divergent evolution
eon
epoch
era
evolution
evolutionary radiation
extinction
fecundity
fixed
fossil
fossil record
founder effect
gene flow
gene pool
genetic drift
genetic isolation
geographical isolation
geological timescale
Gondwana
habitat fragmentation
hybrid inviability
hybrid sterility
macroevolution
mass extinction
microevolution
molecular homology
natural selection
negative selection
parallel evolution
parapatric speciation
period
polyploidy
positive selection
selection pressure
selective agent
sexual selection
single nucleotide polymorphism (SNP)
speciation
stabilising selection
sympatric speciation
temporal isolation
viability
viable offspring

KEY QUESTIONS

Retrieval

1 Identify the period of the evolutionary timescale when multicellular animals first appeared and diversified.

A Silurian
B Jurassic
C Cambrian
D Ediacaran

2 According to the information in Table 9.1.1 on page 437, vascular land plants:

A first appear in the Paleogene
B are present in the Cambrian and radiate in the Ordovician
C first appear in the Silurian and are destroyed in the Cretaceous
D first appear in the Silurian and radiate in Mesozoic–Cenozoic radiation

3 *Drosophila* flies (fruit flies and vinegar flies) normally have red eyes. The white eye mutation has occurred independently in several species of *Drosophila* flies. It has also occurred in other types of fly. Distinct species carrying the white eye mutation can be categorised as an example of:

A divergent evolution
B parallel evolution
C convergent evolution
D parallel speciation

4 Select the most likely explanation for the longevity of the coelacanth (shown below), a taxonomic group that has existed since the Devonian.

A They produced many offspring.
B They did not have any predators.
C They did not compete with each other for food.
D They successfully reproduced in a relatively stable habitat.

5 Choose the process that is the source of new genetic material.

A gene flow
B mutation
C genetic drift
D natural selection

6 Natural selection acts directly on:
- **A** each allele
- **B** the genotype
- **C** the phenotype
- **D** the entire gene pool

7 Natural selection acting over time on a population of wild mung beans is likely to result in:
- **A** mutations that delete alleles
- **B** extinction due loss of alleles
- **C** a relative change in allele frequencies
- **D** genetic drift that increases allele frequency

8 Choose the statement that best describes macroevolution.
- **A** changes in allele frequency in a gene pool of a species
- **B** new traits formed by somatic cell mutations in an individual
- **C** formation of a new population that produces viable fertile offspring
- **D** change in allele frequencies in populations giving rise to new genera and taxonomic groups above species level

Comprehension

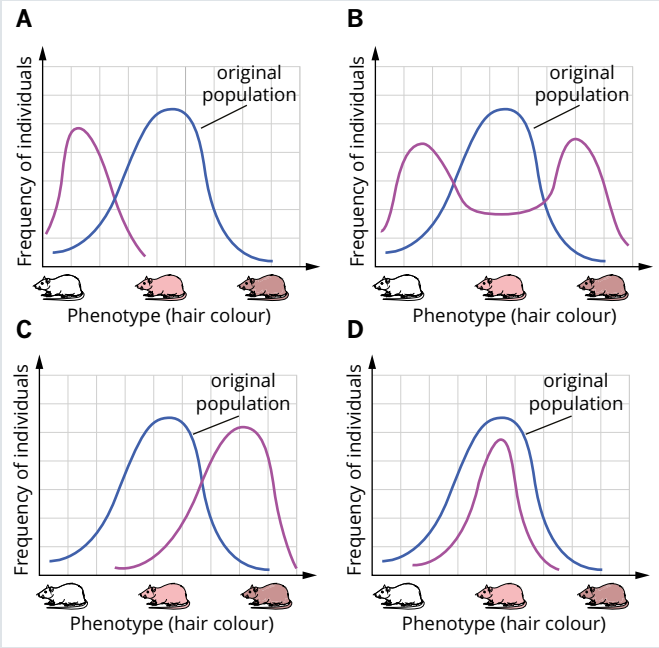
9 The long-nosed bat (shown below), lives near the border of Texas and Mexico. Its main food source is nectar and pollen produced by agave plants. The bats have specialised feeding structures that allow them to reach the nectar. As they eat the nectar the bats become covered in pollen, which they then transfer to another agave plant, thereby pollinating it. Many of these plants not only have flowers that are especially shaped for the feeding structures of the bats but they also flower at night when the bats are most active.

The relationship between the agave plant and the bats has occurred as a result of:
- **A** coevolution
- **B** parallel evolution
- **C** divergent evolution
- **D** convergent evolution

10 Explain how evolutionary radiation contributes to macroevolution.

11 List and describe two factors that can contribute to mass extinction.

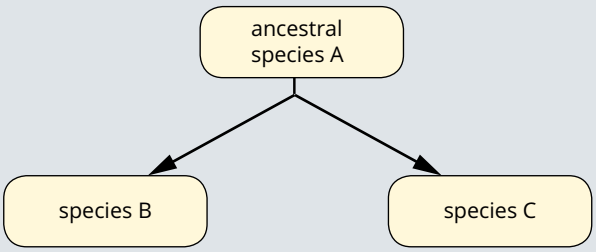
12 The original rock pocket mouse is thought to have been the same colour phenotype as the current light-coloured mice that inhabit sandy, granite locations.
- **a** Choose the diagram that represents the mode of selection leading to the dark phenotype pocket mice living on the lava flows.
- **b** In relation to the alleles determining the dark phenotype, name the type of selection occurring.
- **c** In relation to the dark pocket mouse, name and describe the mode of phenotypic selection.



13 Consider the simple model of speciation illustrated below.

a Use your understanding of variation, isolating mechanisms and natural selection to explain how an ancestral species might evolve into two new species.

b Identify the main criterion applied to determine whether two organisms belong to the same species.



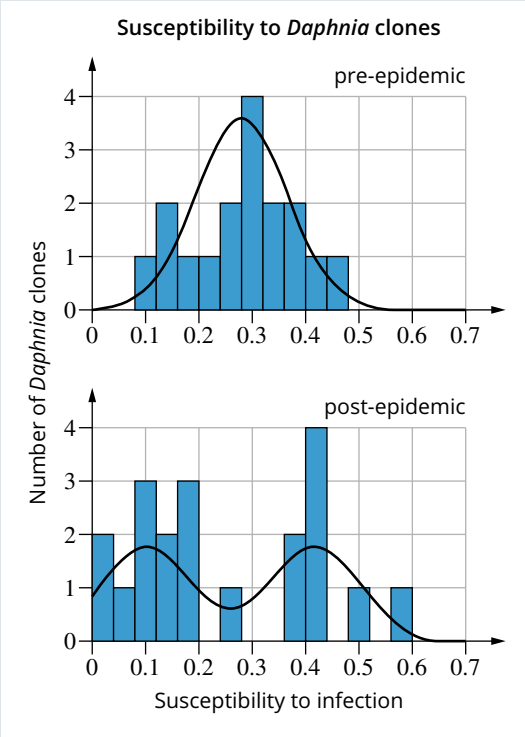
14 Compare temporal and spatial isolation mechanisms.

15 Use an example to explain the association between evolutionary radiation and macroevolution.

Analysis

16 Derive plausible evolutionary processes (genetic and selection) that may underlie the rapid increase in the number of mammalian fossils from the Paleocene epoch onwards when dinosaurs became extinct.

17 *Daphnia dentifera* is a small freshwater crustacean that inhabits lakes in North America. Researchers studied a population of *D. dentifera* during an epidemic infection with a parasitic yeast, *Metschnikowia bicuspidata*. The fungus clogs the gills, lungs and heart of the crustacean.



The researchers hypothesised that the infection would lead to phenotypic selection for animals resistant to infection. The study measured susceptibility to infection of *D. dentifera* clones before and after the epidemic outbreak of the yeast infection. The results are shown in the graph in the left column, where a low value means low susceptibility to infection and a high value means high susceptibility.

a Compare the pre- and post-epidemic data.

b Conclude whether phenotypic selection has occurred and, if so, what type. Justify your answer.

c Judge the researchers' hypothesis. Justify your answer.

d The researchers proposed that these animals experience a trade-off between susceptibility to infection, and body size and fecundity (reproductive success). Large *Daphnia* produce more offspring (they have higher fecundity); however, they are also more susceptible to infection by this yeast. If the researchers are correct, and if genomic analysis became available for genes associated with body size, predict the impact of epidemic yeast infection on the allele frequency for large body size in the *Daphnia* population.

18 Stoneflies, in the insect order Plecoptera, live around streams and lakes. A population of the stonefly species *Leuctra hippopus*, which inhabits a set of rapids in central Norway, is thought to be in the process of speciation. The isolation mechanism(s) leading to the divergence in the population is under study. This population varies from neighbouring populations in the following characteristics:

- Adults emerge from the nymph stage about a month earlier and live for a shorter period.
- They have distinct morphology, e.g. varied body shape.
- They are flightless.
- They are genetically distinct (based on AFLP and SNP allele data).

a Evaluate the researchers' conclusion that temporal isolation from other populations of the species is a significant factor in the divergence.

b Based on this information, conclude the type of speciation underway in these stoneflies.

19 The coyote (*Canis latrans*) and the grey wolf (*Canis lupus*) are closely related species and both have a diploid number of 78. The ranges of the two animals overlap. Hybrids have been identified in the wild. The hybrids seem to express more coyote genes than wolf genes, but are bigger and more able to withstand harsh conditions than pure-breed coyotes. Breeding experiments in which female coyotes were crossed with male wolves resulted in viable offspring but showed

reduced fertility, with two out of three pregnancies failing. Hybrids, however, are able to reproduce with a lower success rate than pure breeds of either species. Genetic studies have shown that the eastern coyote population is almost certainly the result of such matings in the past (more than 200 years ago). Based on this information, identify a term that this situation is most closely related to.

20 Four plants have similar leaf shape but plant 4 has a different colour and was proposed to be distantly related to the others. A section of DNA from each species is aligned.

a Copy and complete the data matrix for the sequence alignment.

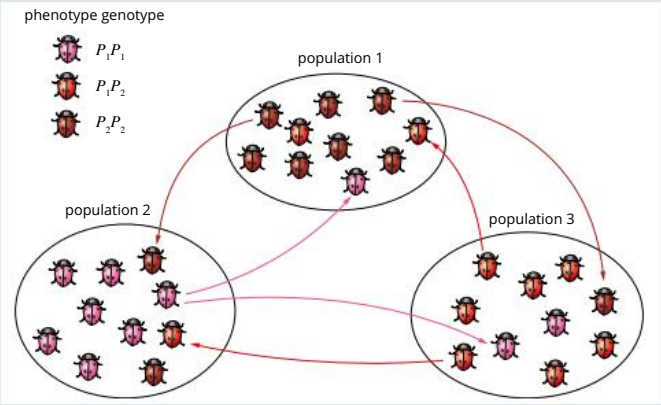
b From this data infer which two species are most distantly related. Justify your answer.

Multiple alignment

```
P1 AGGCCAAGCCATAGCTGTCC
P2 AGGCAAAGACATACCTGACC
P3 AGGCCAAGACATAGCTGTCC
P4 AGGCAAAGACATACCTGTCC
```

	Plant 1	Plant 2	Plant 3	Plant 4
Plant 1	–			
Plant 2		–		
Plant 3			–	
Plant 4				–

21 View the schematic of a hypothetical insect population below. Each population started with one coloured phenotype: population 1 with all dark, population 2 with all light and population 3 with all intermediate.



a Name the process that has occurred to result in the phenotype distribution shown in the diagram.

b Describe the importance of this process in natural selection.

c Identify the population with the highest frequency of the P_1 allele. Show your calculation for this population.

d Predict the consequence of environmental change leading to negative selection of the P_2 allele in population 2. Refer to phenotype, genotype and allele frequency in your answer.

e A flood completely isolated population 3 from the others and 75% of the population died. After the flood subsided, environmental conditions stabilised and returned to the previous conditions. Over time, the predominant phenotype was dark. Name the process and describe the key points.

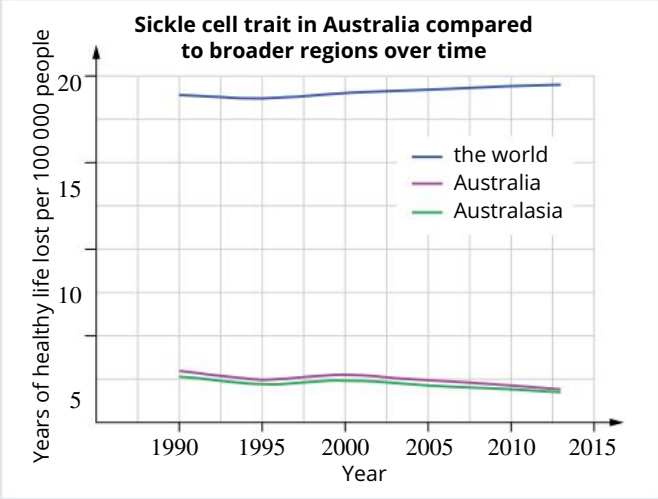
22 The varying degrees of the sickle-cell disease are determined by the following combinations of alleles in individuals.

Normal haemoglobin	Sickle-cell trait	Sickle-cell disease
Hb^AHb^A	Hb^AHb^S	Hb^SHb^S

Heterozygotes show greater resistance to the mosquito-borne parasite *Plasmodium falciparum*, which causes malaria, than do individuals with normal haemoglobin.

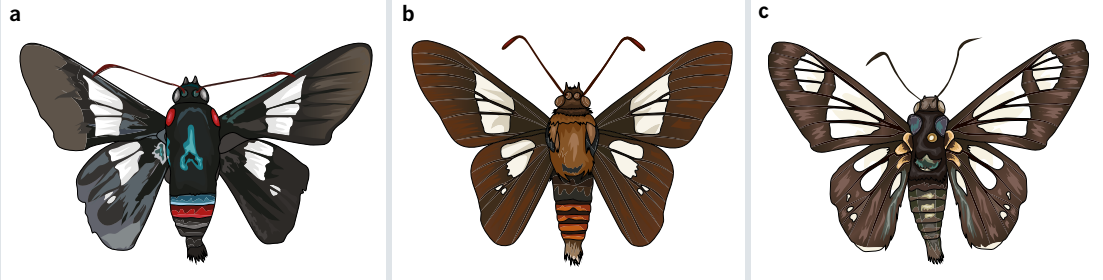
a Identify the process that caused the Hb^S allele to appear.

b Identify the process resulting in the increased heterozygote frequency in regions where malaria is epidemic.



c Describe qualitatively the prevalence of the Hb^S allele in Australia compared to the global prevalence.

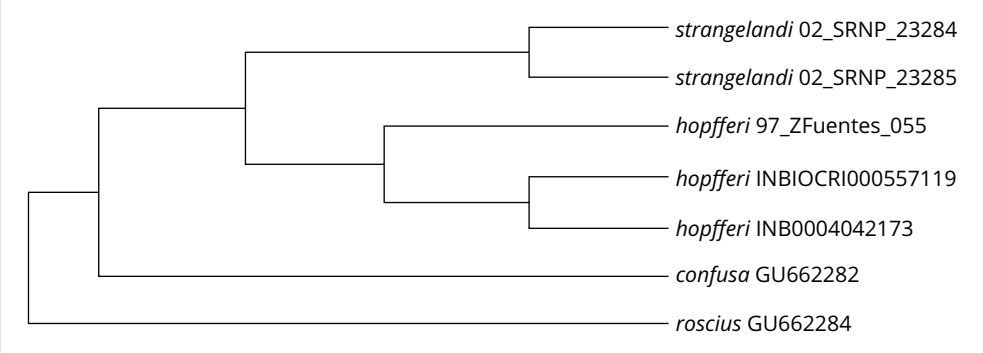
23 A new species and phylogeny of *Oxynetra* butterfly in Costa Rica was identified through DNA barcoding. *Oxynetra* butterflies vary in their body colour pattern and presence of stripes.



Oxynetra butterflies: (a) *Ox. strangelandi*, (b) *Ox. hopfferi* and (c) *Ox. confusa*.

species	
Ox. strangelandi	AATAATACTCATATGTTTACTTTTCTTCATCTAAACCTTTGTTATTCATGCTTTCTCATCTTAAA–
Ox. strangelandi	AATAATACTCATATGTTTACTTTTCTTCATCTAAACCTTTGTTATTCATGCTTTCTCATCTTAAAA
Ox. hopfferi	AATAGTATTCTCATACTCACTTCGCTTTACTCAAACCTTTGTTATTCATGCCTCCTCATCTCAGAA
Ox. hopfferi	AATAGTATTCTCATACTCGCTTCGCTTTACTCAAACCTTTGTTGTTCATGCCTCCTCATCCCAGGA
Ox. hopfferi	AATAGTATTCTCATACTCGCTTTGCTTTACTCAAACCTTTGTTGTTCATGCCTCCTCATCTCAGGA
Ox. confusa	ATCGACGTCTATATGTACATACCTCCTTCTTTAAGTATTCACCATTTGTATTTTCTTTCTTAAAAT
Ol. roscius	GATAATGTTCATGCGAATATATTTTTACCTTTGTATACATGTTGCCTGCGAACTTATATTCAGATT
ancestor	ATTAATGTTCATATGTACATATCTCTATCTTTAAATATTTGTTATTTGTGCATTCTTATTCAAAAT

The barcode region from 1 to 3 specimen samples of the four species of butterflies (*Ox. strangelandi*, *Ox. hopfferi*, *Ox. confusa* and *Ol. roscius*) was used to create the phylogenetic tree below.



Identify the features in the DNA sequence that:

a enable identification of *Ox. strangelandi* as a separate species

b place *Ox. hopfferi* as the most closely related butterfly to *Ox. strangelandi*

c show *Ox. confusa* to be more distantly related

24 Polydactyly is a common genetic condition in Chinese and European chicken breeds.
A regulatory gene with two alleles, *A* and *C*, has been identified in association with the trait.

a Use the following equation to calculate the frequency of the *C* allele in:
- **i** Silkie chickens with polydactyly
- **ii** Silkie chickens without polydactyly
- **iii** Houdan chickens with polydactyly
- **iv** Houdan chickens without polydactyly

$$\text{allele frequency} = \frac{2(\text{number of homozygotes}) + (\text{number of heterozygotes})}{2(\text{total number of individuals})} \times 100$$

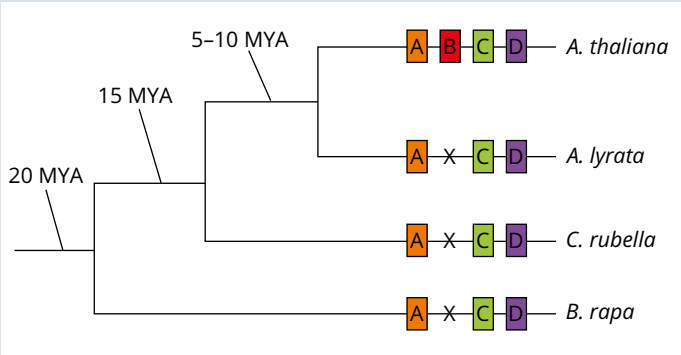
Genotypes of five chicken breeds in birds with and without polydactyly

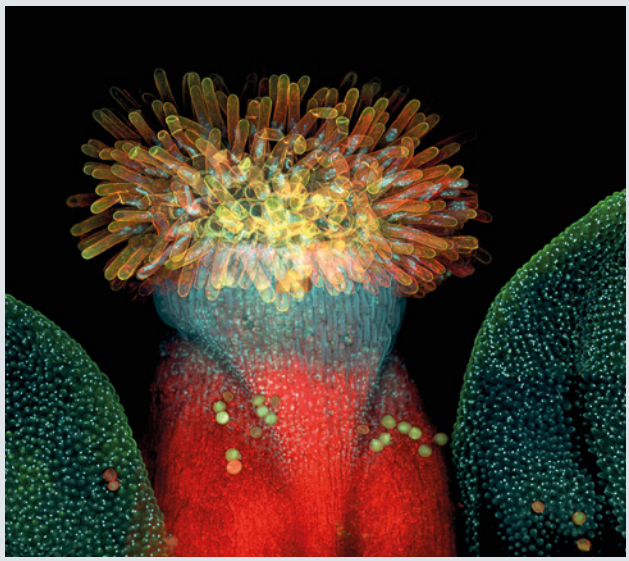
Breed name	Number							Origin
	Total	Polydactyly			Non-polydactyly			
		CC	*AC*	*AA*	*CC*	*AC*	*AA*	
Beijing-You	236	0	51	4	181	0	0	China
Silkie	110	0	39	62	9	0	0	China
Jiningbairi	38	0	18	0	20	0	0	China
Houdan	86	0	43	0	43	0	0	France
White leghorn	96	0	0	0	96	0	0	Italy

b Consider whether the genotype data implies that there is more than one genetic pathway to polydactyly.

c Describe how this case illustrates parallel evolution.

25 Comparative genomics analysis of four closely related species of the cress plant *Arabidopsis* identified four genes (*A*, *B*, *C* and *D*) that arose by gene duplication. The figure below shows a phylogeny based on these duplicate genes.





Fluorescence image of the *Arabidopsis thaliana* flower

a Identify the genetic change that corresponds to divergence of *A. lytra* and *A. thaliana*.

b Predict how this new gene, formed by gene duplication, can contribute to speciation.

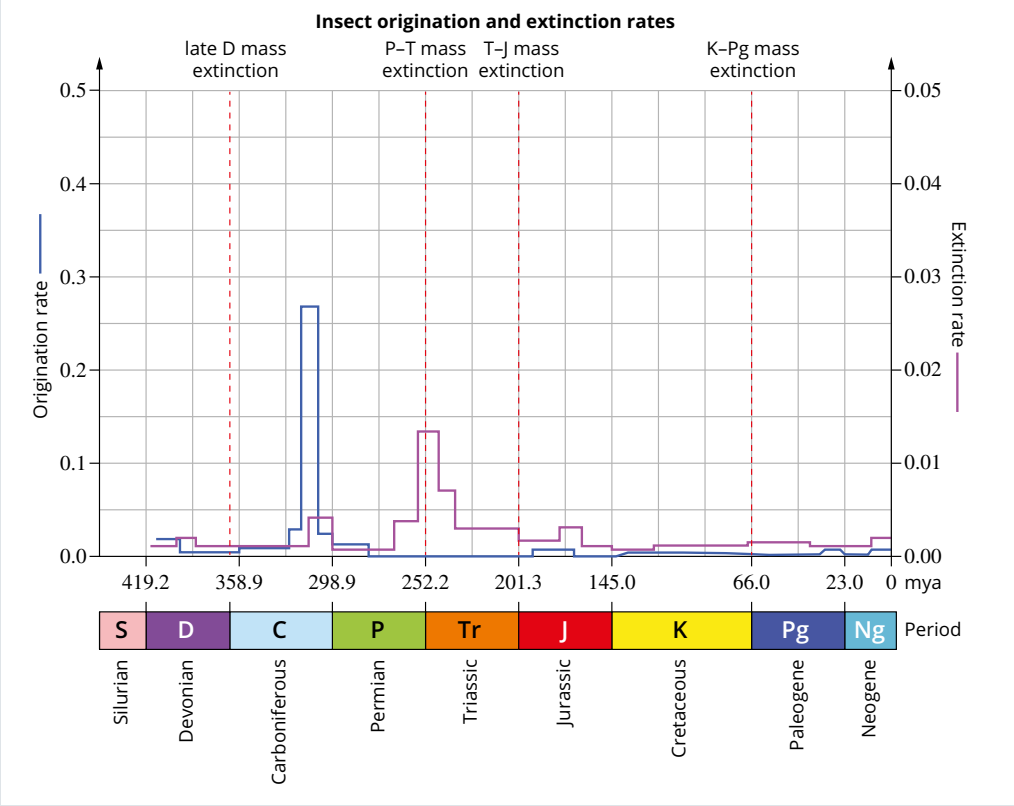
Knowledge utilisation

26 The graph illustrates the rate of insect order appearance in the fossil record (origination rate) and the extinction rate from the Silurian to the Neogene periods.

a Determine a period of radiation of insect orders.

b Explain why this data represents evolutionary radiation rather than speciation.

c Identify the event suggested to have occurred at 252.2 mya.



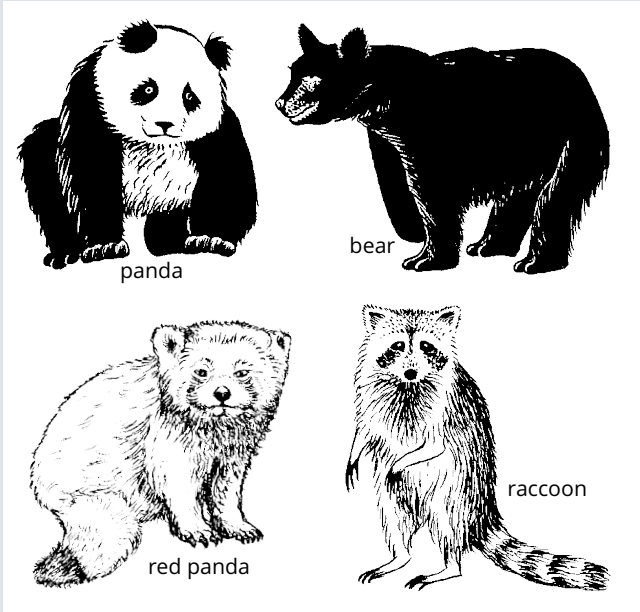
27 The red panda (*Ailurus fulgens*) was once thought to be closely related to the giant panda (*Ailuropoda melanoleuca*). Both species occur in China, they are members of the order Carnivora, and both animals eat bamboo. They also share an unusual morphological feature, a pseudothumb, where a wrist bone is elongated. The pseudothumb is used for grasping bamboo and climbing trees. However, initial genetic comparison suggested that red pandas are more closely related to racoons, and giant pandas are more closely related to bears.

a Identify the reasons for the traditional view that the red and giant pandas were related.

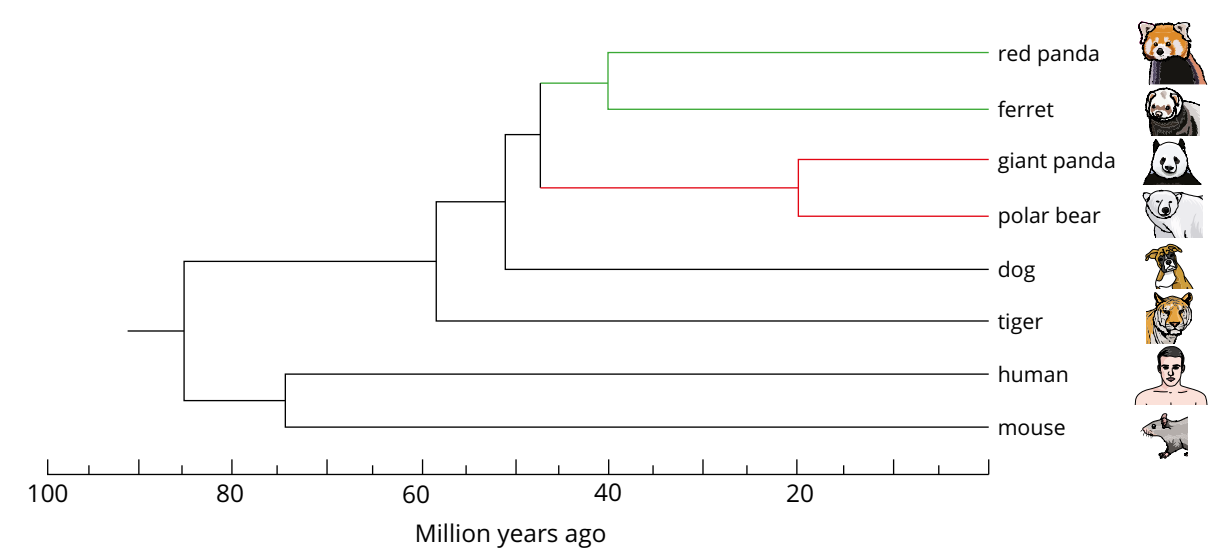
b Identify whether divergent or convergent evolution is suggested by the comparison of the following pairs of animals:

i red panda and giant panda

ii red panda and raccoon



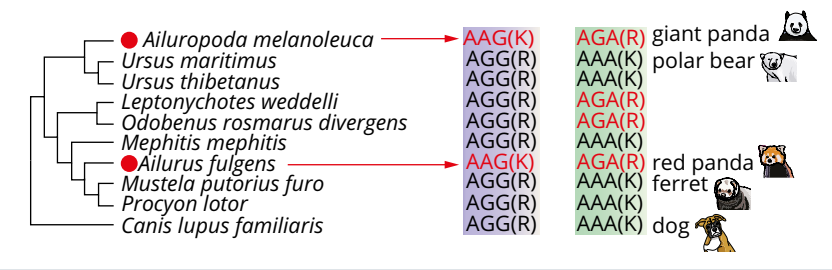
Comparative genomics now provides more information. Whole genome sequencing of the two pandas and several other carnivores produced the phylogenomic tree below.



c Based on this phylogeny, identify the closest proposed relative of:
- **i** giant panda
- **ii** red panda

d Explain if the new data requires you to revise your conclusion regarding divergent or convergent evolution between the two pandas.

The genomic study identified genes involved in regulating limb development, hand and foot development that could be the genetic basis of the pseudothumb, which is absent in the other animals compared. Two DNA triplets of one gene, *DYNC2H1*, are shown below. The letters R and K are the abbreviations for the amino acid encoded by the triplet, R (arginine) and K (lysine).



e Compare the DNA sequence for the amino acid between the two pandas and between pandas and the other species shown.

f Deduce the effect of the changes on the pseudothumb phenotype from the information provided.

REVIEW QUESTIONS

Topic 1 DNA, genes and the continuity of life

TR 4.1 TR 4.2

Multiple choice

1 Complete this statement: A DNA molecule consists of two strands in which:

A the percentage of adenine is the same in each strand
B the percentage of adenine is the same as that of thymine in each strand
C the percentage of adenine is the same as that of uracil in the whole molecule
D the percentage of adenine is the same as that of thymine in the whole molecule

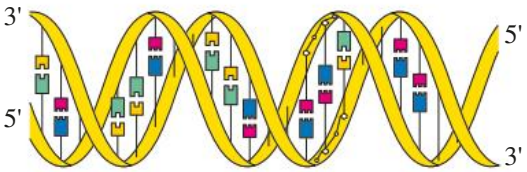
2 Identify the laboratory technique that is used to separate fragments of DNA.

A karyotyping
B genetic screening
C gel electrophoresis
D polymerase chain reaction

3 Consider the structures shown below.

Structure A

Structure B



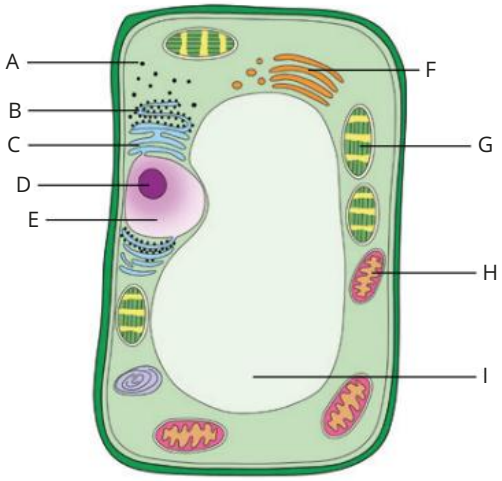
Structure C

Structure D

Identify the alternative that correctly names the type of nucleic acid forming each structure.

	Structure A	Structure B	Structure C	Structure D
A	ribosomal RNA	messenger RNA	DNA	transfer RNA
B	transfer RNA	DNA	messenger RNA	ribosomal RNA
C	transport RNA	DNA	messenger RNA	ribosomal RNA
D	messenger RNA	DNA	ribosomal RNA	messenger RNA

4 Examine the following diagram of a cell.

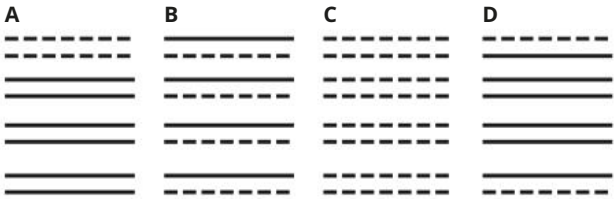


Identify the organelles which contain DNA.

A organelles D, E and F
B organelles B, C and D
C organelles D, G, I and F
D organelles D, E, G and H

5 DNA replication is a semi-conservative process. In order to demonstrate this, a piece of radioactively labelled DNA was allowed to replicate through two cycles. The radioactively labelled piece of DNA is shown below.

The new strands of DNA are not radioactive and are shown as solid lines. Infer which diagram shows the DNA after two replication cycles.



6 Recall which of the following does not contribute to variation in future offspring.

A recombination
B germline mutations
C somatic cell mutations
D independent assortment

7 Interpret the flow diagrams and identify which of the following correctly lists the sequence of the events of meiosis.

A crossing over occurs → chromosomes replicate → cytokinesis occurs
B chromosomes replicate → haploid daughter cells form → chromatids separate
C chromatids separate → chromosomes line up at the equator → cytokinesis occurs
D pairing of homologous chromosomes → chromosomes replicate → crossing over occurs

8 Consider this strand of DNA:

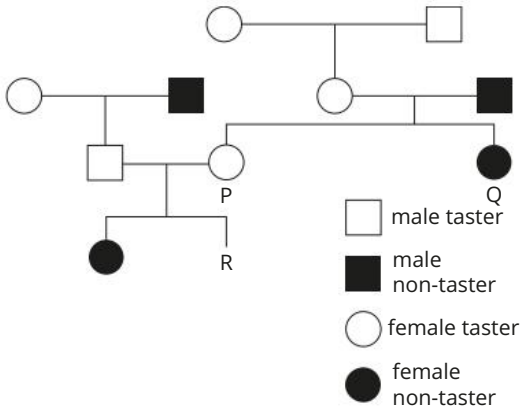
TAACCTAAG

Conclude which one of the following sequences is the corresponding strand of mRNA.

A UTTGGUTTC
B ATTGGATTC
C UAACCUAAG
D AUUGGAUUC

Use the information below, and in the pedigree chart above right, to answer Questions 9, 10 and 11.

The ability to taste a particular chemical, PTC, is genetically controlled by one gene. The pedigree chart shows the transmission of this gene in a family.



9 Determine the mode of inheritance of the tasting allele.

A Y-linked
B X-linked recessive
C autosomal dominant
D autosomal recessive

10 Determine the probability that child R is a taster.

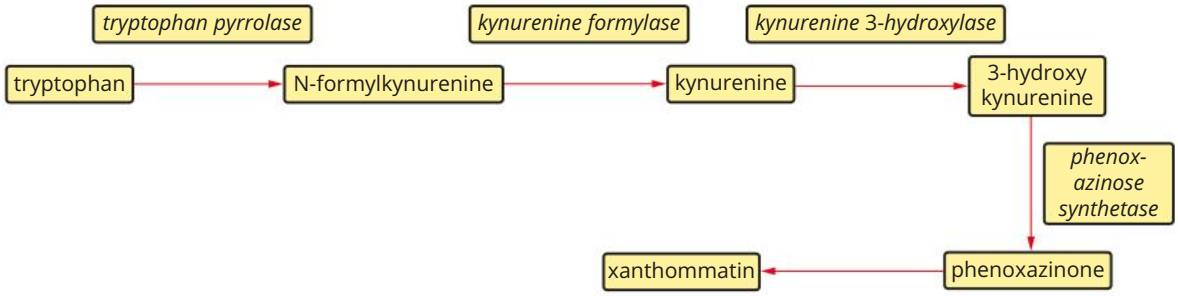
A 0.13
B 0.25
C 0.38
D 0.75

11 Infer the genotypes of females P and Q.

	P	Q
A	*tt*	*tt*
B	*tt*	*Tt*
C	*Tt*	*tt*
D	*Tt*	*Tt*

---

12 An example of a biochemical pathway is shown below. This pathway produces a brown pigment in the eyes of *Drosophila*.



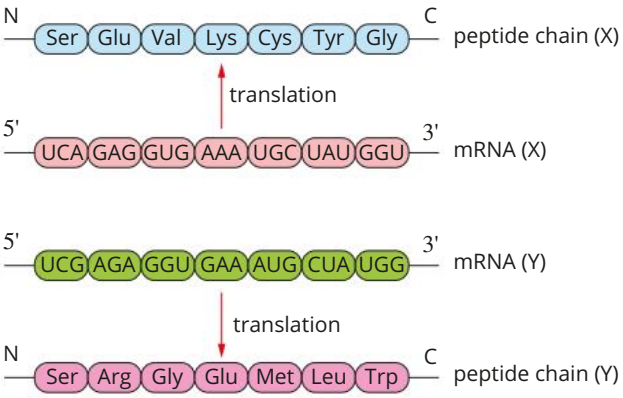
Determine the consequences of a mutation in the gene coding for kynurenine formylase.

A an accumulation of kynurenine but no production of N-formylkynurenine
B an accumulation of N-formylkynurenine and kynureine but no production of phenoxazinone
C an accumulation of N-formylkynurenine and no production of kynurenine or any other product
D an accumulation of N-formylkynurenine and phenoxazinone but no production of xanthommatin

13 Cancer results from at least two failures of normal cell behaviour. First, the cell undergoes a mutation, which causes an abnormally high rate of cell division. Then the abnormal cells fail to undergo apoptosis. An important protein in this process is called p53. When active, p53 stops mitosis at an early checkpoint if DNA damage is detected, so DNA repair can occur. If repair is not possible, p53 initiates apoptosis. The protein p53 affects the expression of the genes for two proteins important to apoptosis. These proteins are Bax, which promotes apoptosis, and Bcl, which inhibits it. The Bax protein forms a protein channel in the membrane of a mitochondrion through which cytochrome *c* can escape, initiating programmed cell death. The Bcl protein blocks that channel. Infer which of the following statements would not be true to say of this process in healthy cells.

A The protein p53 stops transcription of Bcl.
B The gene coding for p53 is a regulatory gene.
C When a cell has severe DNA damage, p53 would normally increase the transcription of Bax.
D The pathway to apoptosis initiated by p53 involves the formation of protein channels in the mitochondrial membrane using Bax proteins.

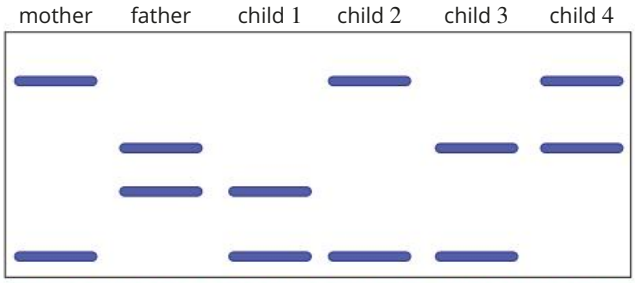
14 The diagram below shows a mutation. Peptide chain (X) is the normal chain and peptide chain (Y) is the mutated version.



Identify the type of mutation.

A a point mutation
B a block mutation
C a silent mutation
D a frameshift mutation

15 The following DNA profile was obtained for a man, a woman and their four children.



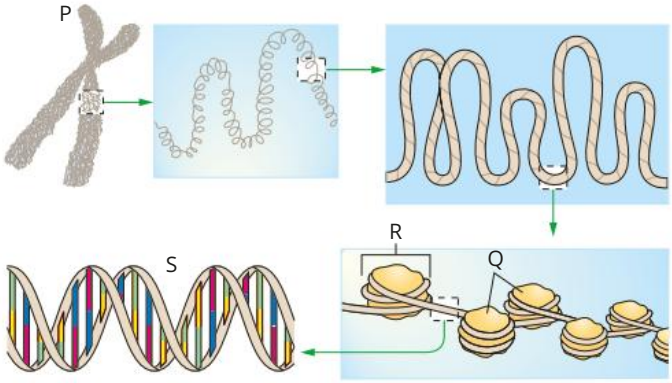
Identify which child is least likely to be the biological offspring of the father.

A Child 1
B Child 2
C Child 3
D Child 4

Short answer

16 Distinguish between the roles of helicase and DNA polymerase during the cell cycle.

17 The figure below shows the relationship between chromosomes, DNA and genes.



a Identify structures P, Q, R and S.
b Using the diagram, outline how DNA is packaged into chromosomes.

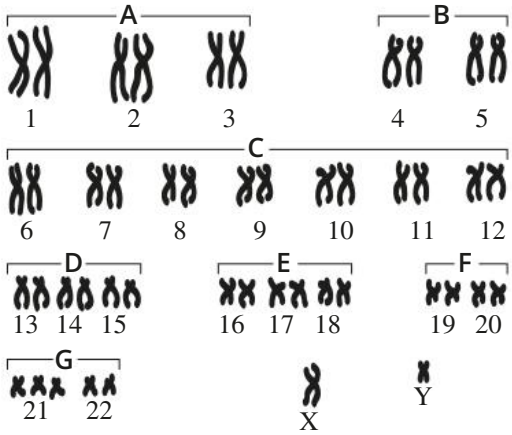
18 **a** Distinguish between genes and the genome.
b Distinguish functional genes from regulatory genes.
c Outline the structure of eukaryotic genes.
d Using the *lac* operon in *E. coli* as an example, explain how gene regulation by transcriptional factors expressed by regulatory genes can occur.

19 In bees and wasps (and some mites, rotifers and beetles), the males of the species are haploid. The males develop from unfertilised eggs, yet the sons of the same female are not all identical.

a Explain, with reference to the processes occurring during egg formation, how the sons can be genetically different.
b Draw diagrams to show the different genotypic outcomes for two genes next to each other on the same chromosome during the process of egg formation, where crossing over occurs and when it does not occur.
c Explain whether the sperm produced by a male bee is genetically varied.

20 Outline ways in which the environment and interaction of DNA with other molecules (epigenetics) may affect the expression of a gene in an individual and hence its phenotype. Provide an example in your answer.

21 The picture below shows the karyotype of an individual.

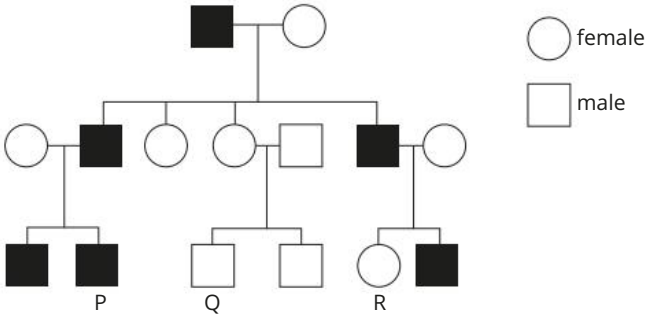


a **i** Identify the abnormality present in this karyotype.
ii Name the term used to describe all similar conditions.

b Explain how such a condition could arise.

22 Distinguish between inheritance of multiple alleles and polygenic inheritance. Give an example for each.

23 The pedigree chart below shows the hypothesised inheritance of oligospermia. Oligospermia results in low sperm count and sometimes morphological changes to sperm, making fertility difficult. Shaded individuals possess oligospermia.



a State the type of inheritance shown.

b Assign an appropriate symbol and state the genotype for individuals P and R.

c Calculate the probability of any of individual Q's children having oligospermia. Show your working using a Punnett square.

24 **a** List three factors that can cause epigenetic effects.

b Alzheimer's disease results from damage to brain tissue caused by the formation of abnormal proteins. It has been shown through familial studies to have a genetic component, but identical twin studies have shown that just because one twin develops Alzheimer's there is no certainty that the other will as well. Explain how epigenetics can account for this.

25 In humans, the adenomatous polyposis coli (*APC*) gene is located on chromosome 5. *APC* controls cell division and is also known as a tumour suppressor gene. Mutations of *APC* will cause a genetic disease called familial adenomatous polyposis (FAP).

a Explain whether FAP is a sex-linked genetic disease.

b Half of the gametes produced by a person with FAP have an *APC* gene with the mutation. State whether FAP is a dominant or recessive phenotype. Justify your choice.

c A male who is heterozygous for FAP and an unaffected female are planning to have children. Predict the possible phenotypes and genotypes of the children, showing your working.

26 The body plan of insects is that they have a head, a thorax and an abdomen. The thorax is formed from three embryonic segments: T1, T2 and T3. In most insects, each segment grows one pair of legs. Segments T2 and T3 also grow a pair of wings. Flies, however, have only one pair of wings, which grow on T2. The ultrabithorax (*Ubx*) gene controls this situation. *Ubx* is a master regulator gene that encodes a transcription factor that represses the formation of wings on T3 in flies.

a **i** Define transcription factor.
ii Describe the likely effect of a non-lethal mutation that renders the *Ubx* gene completely non-functional in a fly.

b *PAX-6* is a transcriptional factor that binds to DNA and is used during embryonic development of eyes. It is found in many animals and the human *PAX-6* gene can be inserted into flies and result in correct eye formation in flies even though the eye structure is different. Argue how *PAX-6* can result in the formation of functional eyes in both humans and fruit flies.

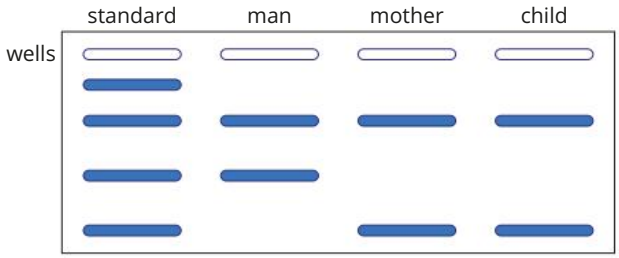
c Identify another example of a transcriptional factor that regulates body plan.

27 A man was not certain that a child was his biological offspring. He decided to have his child's DNA tested to be certain.

a Identify a possible source of the DNA that could be used.

b Draw a flow chart detailing the process scientists would use to produce enough DNA to create a DNA profile of the man and child.

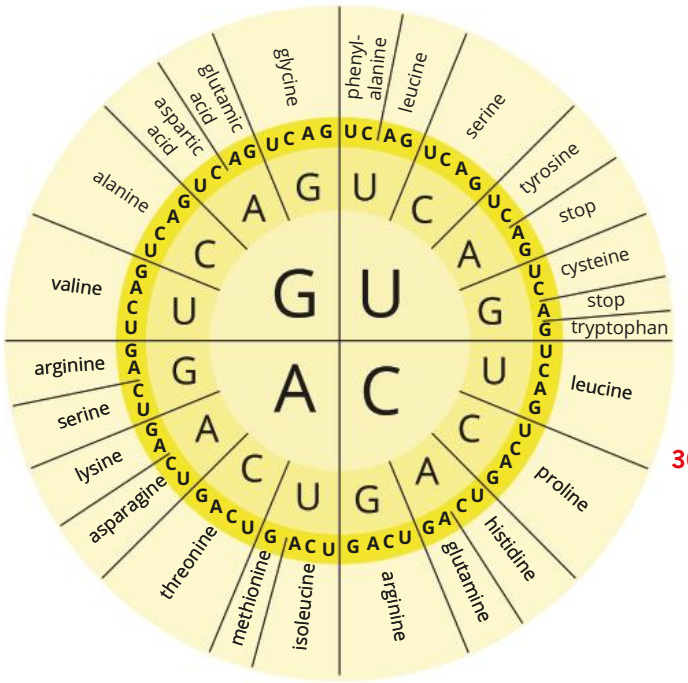
c A DNA profile was made using the amplified DNA from the man, mother and child in question. The profile is shown below.



i Explain why standards are required.

ii Explain whether the man is likely to be the child's biological father.

28



a State the sequence of amino acids in the polypeptide synthesised using the following piece of DNA: ATGTCAAAATGT. Use the diagram above and show each of the steps taken to decode the sequence.

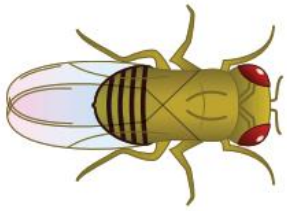
b Exposure to radiation causes a change in the DNA resulting in the deletion of the second T.

i Name the type of mutation caused by the deletion of the T.

ii Explain how this would result in a change in the polypeptide formed.

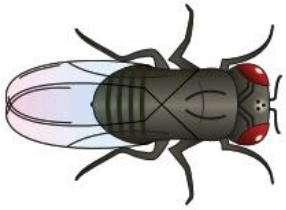
iii State the sequence of amino acids formed from the mutated DNA.

29 The fruit fly, *Drosophila melanogaster*, is commonly used for genetic studies. One reason for this is that sex determination in *Drosophila* is the same as humans. Females are XX and males are XY.

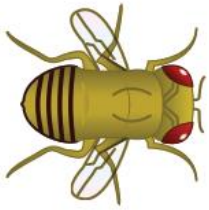


wild type

In *Drosophila melanogaster*, a mutation results in offspring that have an ebony (black) body while another mutation results in small wings. These are called vestigial wings. The normal version of the fruit fly is usually called the wild type.



ebony body



vestigial wings

The gene for body colour is on chromosome 3 and the gene for wing size is on chromosome 2. A geneticist took a fly homozygous for wild type at both the body colour and wing shape locus and crossed it with a fly that was homozygous for ebony body and vestigial wings. All of the F_1 offspring were wild type.

a Explain whether ebony body and vestigial wings are dominant or recessive.

b i Show a test cross involving one of the F_1. Clearly identify the genotypes and phenotypes of the parents and offspring.

ii Identify the proportion of the offspring which will possess only one recessive phenotype.

30 The table below shows details of certain human genes.

Selected human genes

Gene and gene product	Gene size (kb)*	mRNA size (kb)	Number of introns
HBB gene (beta globulin)	1.5	0.6	2
ALB gene (albumin)	25.0	2.1	14
PAH gene (phenylalanine hydroxylase)	90.0	2.4	12
CFTR gene (cystic fibrosis transmembrane conductance regulator)	250.0	6.5	26
DMD gene (dystrophin)	2500.0	17.0	70

*kilobase pairs

a Calculate the average size of introns for the albumin gene. Show your working.

b Describe the relationship between gene size and number of introns.

c Calculate the maximum number of amino acids that could be produced by translating dystrophin mRNA.

31 Human growth hormone (HGH) is one important pharmaceutical produced using recombinant DNA technology. The process uses bacteria to produce a pure, safe supply of HGH (somatotropin).

a mRNA is usually used in order to determine the sequence of genes for use in recombinant DNA techniques using bacteria, such as in the production of HGH. Create a flowchart showing the steps involved in producing HGH for human use.

b Explain why mRNA is used to obtain the gene sequence for HGH rather than DNA.

c Restriction enzymes are used to cut DNA. They identify sequences of DNA and cut those sequences in specific patterns. Three restriction enzymes and their recognition sequences and cutting sites are shown below.

Enzyme 1	Enzyme 2	Enzyme 3
CT | TCCT	GG | GCCC	GAT | ACT
GAAG | GA	CCCG | GG	CTA | TGA

Explain which restriction enzyme should be used to cut open a plasmid that has the sequence shown below:
ATTGAGGTACCGATACTTGACCTGATCACGGGCCCCG
TAACTCCATGGCTATGAACTGGACTAGTGCCCGGGGC

32 In a laboratory, a transformation experiment was performed to evaluate which bacterial strain would be best for transformation with a plasmid called pGK12. The pGK12 plasmid contains a gene conferring erythromycin (an antibiotic) resistance. This was done to decide which bacterial strain would be most appropriate for use in human protein production.

Transformation success is given by the formula:

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on plate}}{\text{Amount of DNA spread on plate in } \mu g}$$

Bacteria were exposed to 2–4 μg of DNA, and the number of colonies was determined after 24 hours of incubation. The results are shown in the table below.

Bacterial transformation

Species	DNA concentration (μg)	Number of transformed colonies ($\times 10^3$)
Enterococcus faecalis	2	4.3
	3	7.9
	4	5.2
Lactobacillus casei	2	3.9
	3	6.2
	4	5.6
Listeria innocua	2	5.2
	3	6.4
	4	4.1
Staphylococcus aureus	2	3.1
	3	3.9
	4	4.5

a Calculate the transformation efficiency of the various bacterial species at the range of concentrations of plasmid given.

b Propose which bacterium and concentration of DNA should be used to produce human proteins. Justify your recommendation.

c In a commercial situation, the decision about which species to use would not be based solely on transformation rate. Propose other information that would be needed in order to make a final recommendation as to which species of bacterium to use for production of human proteins.

Topic 2 Continuity of life on Earth

Multiple choice

1 Which of the following statements most correctly relates evolution and natural selection?

A Natural selection stops evolution.
B Evolution leads to natural selection.
C Natural selection is a mechanism for evolution.
D Natural selection is the mechanism for evolution.

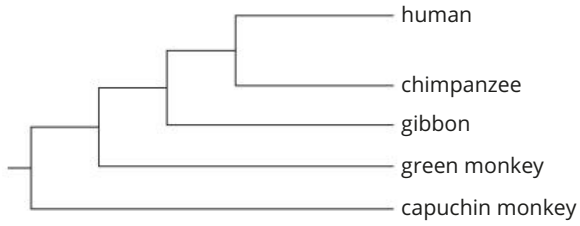
2 The table below shows the differences between the DNA of a human and four other species of primate.

Human DNA compared to four other primates

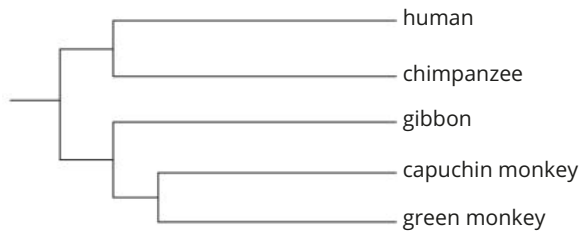
Species tested against human DNA	Difference (%)
human	0.0
chimpanzee	2.4
gibbon	5.3
green monkey	9.5
capuchin monkey	15.8

Select the tree that best shows the relationships portrayed in the table.

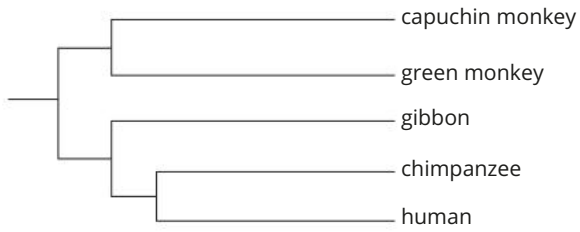
A



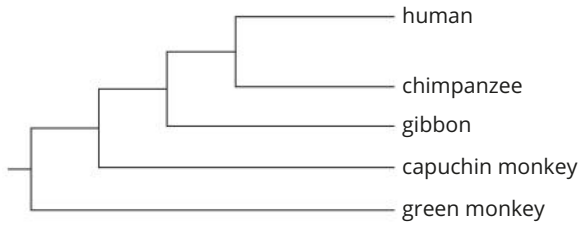
B



C



D



3 The clearwing moth (family Sesiidae) and the yellow jacket wasp (family Vespidae) have very similar morphology. The moth mimics the wasp. Infer the likely type of evolution.

A Divergent evolution as a result of genetic drift.
B Convergent evolution as a result of genetic drift.
C Divergent evolution as a result of natural selection.
D Convergent evolution as a result of natural selection.

4 Select the statement that best describes the history of the Galapagos Island finches from the evidence observed.

A They are the same today as when the ancestral finches arrived on the islands.
B They developed as a result of regular gene flow between the mainland and the islands, along with mixing of populations between the islands.
C They are a result of an evolutionary radiation, where an original colonising population diversified to take advantage of a number of available niches.
D They demonstrate convergent evolution, as they were once distinct species that have grown more similar over time due to adaptation to similar niches and selection pressures.

5 The DNA sequence of part of the mitochondrial DNA for five primate species is shown here.

Part mtDNA sequence for five primates

Species	DNA sequence
Homo sapiens	ACACCATA
Pan paniscus	ACACCATA
Gorilla gorilla	CCACCACA
Pongo abelii	CCACCACA
Nomascus concolor	CCACCATA

Select the statement that is most consistent with the data.

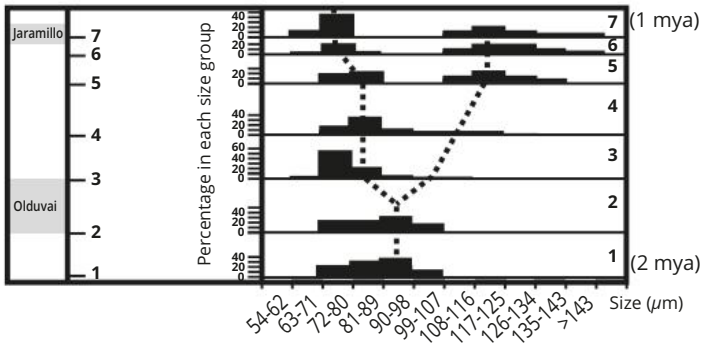
A The data is incomparable and it is invalid to draw a conclusion.
B The sequence of *Pan paniscus* reflects *Gorilla gorilla* more closely than *Nomascus concolor*.
C *Homo sapiens* reflects *Pan paniscus* as equivalently as *Gorilla gorilla* reflects *Pongo abelii*.
D *Nomascus concolor* and *Pongo abelii* sequences are as equivalent as *Gorilla gorilla* and *Pan paniscus*.

6 Select the scenario that is an example of allopatric speciation.

A breeds of dogs that do not interbreed because of physical dissimilarities
B populations of insects that do not interbreed because of different mating seasons
C populations of frogs that do not interbreed because of differences in mating calls
D groups of large, flightless birds that do not interbreed because of their geographical locations

7 The graph below shows the evolution of a group of plankton called radiolarians.

Evolution of radiolarians



As the graph shows, this group of plankton evolved from a single common ancestor into two distinctly separate species. The graph also shows an example of relative dating. Identify the most likely conclusion.

A Fossils in level 5 are older than fossils in level 2.
B Levels 1 to 4 show decreasing diversity in the radiolarian group.
C Fossils in level 1 are between 108 and 143 micrometres in length.
D Levels 5 and 6 show two distinct groups that still belong to the same species.

Use the following information to answer questions 8 and 9.
The closeness of evolutionary relationships between groups of primates can be indicated by the similarity of amino acid sequences in similar proteins. The results of one such analysis, based on the protein haemoglobin, are shown in the table below.

Number of amino acid differences in haemoglobin protein of primates compared to humans

Primate	Number of differences
gorilla	1
gibbon	2
chimpanzee	0
rhesus monkey	8

8 From this data, identify which primates have the most distant relationship to humans.

A gorilla
B gibbon
C chimpanzee
D rhesus monkey

9 Select the alternative that does not apply when discussing the molecular evidence for evolution.

A Related organisms share a greater portion of their DNA.
B Phylogenetic trees are developed using molecular evidence.
C Only DNA can be examined for establishing evolutionary differences.
D The haemoglobin gene is the best gene to use to compare primate species.

10 A species of moth known as Morgan's sphinx moth has an extremely long tongue that has evolved over many thousands of generations. This moth exploits a food resource unavailable to other moths. It uses its long tongue to collect nectar from the flower of a plant called Darwin's orchid. This plant has a very tubular flower so only the long tongue of the moth can reach the nectar. In the process of collecting the nectar, the moth collects pollen and then carries it to other orchids, which it pollinates. Over time, both the flower tubes and the moth tongues have become longer.

Select the alternative that best describes the relationship between the moth and the orchid.

A coevolution
B natural selection
C allopatric speciation
D convergent evolution

11 Select the situation in which microevolution is occurring.

A A new species is formed.
B The allele frequencies of a population change through a period of time.
C An individual's characteristics change in response to factors in the environment.
D The mix of species in a community change due to the extinction of some species.

12 Select the term which most correctly completes the statement.

Polyploidy can result in speciation. Such an event is an example of _____.

A sympatric speciation
B directional selection
C allopatric speciation
D parapatric speciation

13 For some traits (such as birth weight in mammals), natural selection favours individuals that are average and the extremes are selected against. Identify the term used to describe the type of selection involved.

A directional selection
B disruptive selection
C stabilising selection
D diversifying selection

Short answer

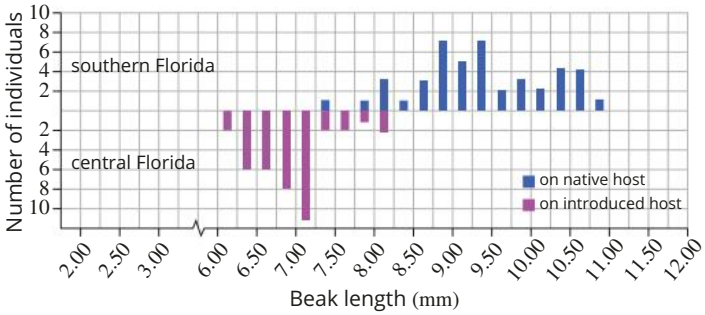
14 In Florida, soapberry bugs (*Leptocoris tagalicus*) feed most effectively when the length of their beak closely matches the depth within the fruits of the seeds they eat. Soapberry bugs fed on the native balloon vine (*Cardiospermum corindum*) until the introduction of the golden rain tree (*Koelreuteria elegans*) from Taiwan in the 1950s. The golden rain tree provided an additional food source for the bugs. The golden rain tree has a flatter fruit shape than the balloon vine.

To investigate the effects of the introduction of the golden rain tree on the evolution of soapberry bugs, the beak lengths of soapberry bug populations in southern Florida feeding on the native balloon vine (*C. corindum*) and populations in central Florida feeding on the introduced golden rain tree (*K. elegans*) were measured.

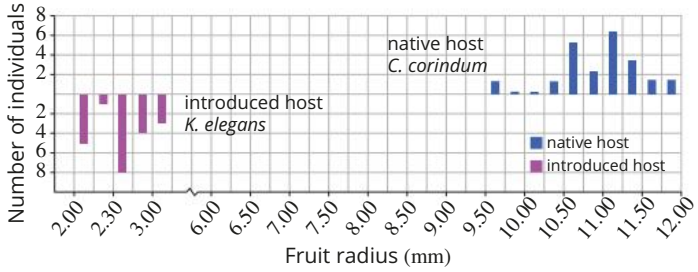
Museum specimens of soapberry bugs collected in the two areas before the golden rain tree was introduced were used as a baseline. These specimens have an average beak length of 8.57 mm.

The results of the comparison are shown below.

Soapberry bug beak length in southern and central Florida



Radius of fruit from the golden rain tree (*K. elegans*) and the balloon vine (*C. corindum*)



a Summarise the results of the comparative study.
b Propose how the change in beak length of soapberry bugs could have occurred.
c Identify the type of phenotypic selection occurring in the soapberry bugs feeding on the golden rain tree.

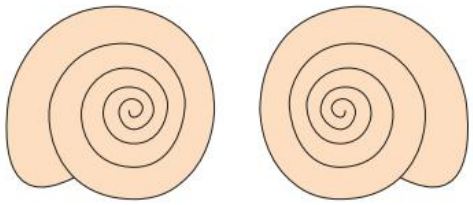
15 The diagram illustrates a section of DNA from a chicken, a quail and a turkey. Asterisks (*) indicate that all three species have the same base at that position. Dashes indicate insertions and/or deletions.

	10	20	30	40	50	60	70
chicken	CAGCCCTTTC	ACCTCCGAAG	GGAATATAGA	GGAGGAG---	---AAGAGAC	CGAGCCCATA	TCGAGAGCCA
quail	**********	**T**T****	***GG*****	*******GAG	GAG*******	***A******	**********
turkey	**********	**********	A**GG*****	*******GAG	GAG*******	***C******	**********

a Based on this sequence, infer which two of the organisms are most closely related. Justify your inference.

b Draw a phylogenetic tree of the three birds based on the data.

16 *Lymnaea* is a genus of mollusc having snail-like shells. Members of *Lymnaea* have shells that coil either left or right. In most individuals, development of this trait is purely genetic, with offspring showing a phenotype that is identical to the maternal phenotype. Occasionally, an individual with the genetics to grow a shell that coils right is influenced by environmental factors that result in this individual growing a shell that coils left (or vice versa).



left-coiled shell right-coiled shell

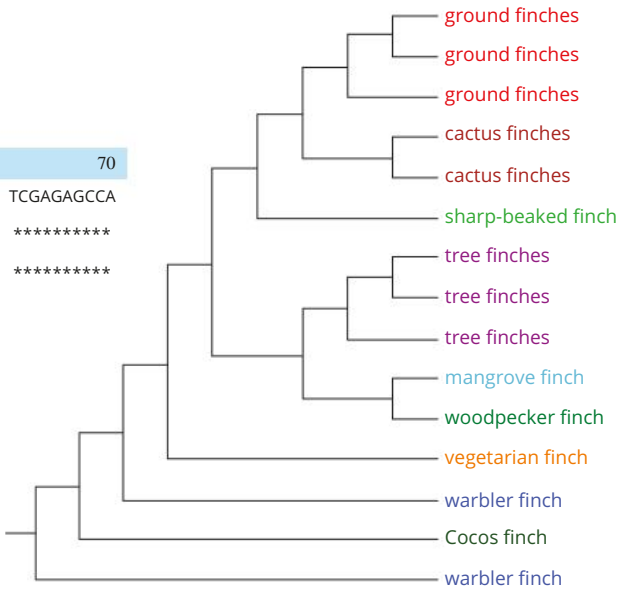
a As a result of physical incompatibility, individuals with shells that coil in the opposite direction are unable to mate. Without the occasional environmental effect on shell growth, this incompatibility could lead to speciation of the two groups. Explain how this could occur.

b Explain why speciation is unlikely as long as the environmental influence continues.

17 Genetic studies show that the Galapagos finches are the descendants of a population that migrated from the mainland of South America, around 950 km away. This is estimated to have occurred around 2.5 million years ago. Finches are small birds, for which even a distance of 100 km is a long way to fly, so the event that brought the finches to the Galapagos must have been one of low probability. Travel between islands, while more frequent than movement from the mainland, would also be uncommon due to the distances involved.

The hypothesised relationship between the various finches living in the Galapagos today is shown in the phylogenetic tree.

Note: Vegetarian finches are also known as herbivorous finches.



a Volcanic activity has occurred over the period since the arrival of the finches, which added 14 islands to the chain. It has been proposed that the climate of the Galapagos has experienced change throughout that time, changing from lush and tropical to cool and dry. It has also been suggested that sea levels have changed, resulting in the isolation of some islands. Explain how climate changes in the Galapagos Islands have may resulted in speciation of the original population of finches to arrive there.

b According to the phylogenetic tree shown, identify which of the Galapagos groups is most closely related to the Cocos finch of the Cocos Islands.

c Describe how niche separation on the Galapagos contributed to speciation of the finches.

18 Describe how natural selection can lead to the evolution of a species in response to environmental change.

19 The peppered moth (*Biston betularia*) is an insect found in English forests. During the 1800s, the Industrial Revolution in England resulted in large amounts of smoke being produced by factories. This polluted the air and created a residue that coated the trunks of trees, making them darker. There are two forms of *Biston betularia:* the melanic form, which is darker in colour, and the paler form, which experiences positive selection when it settles onto paler tree trunks because of lichens growing on the bark.

a In the description above, explain what is meant by the term positive selection.

Before the Industrial Revolution, the light form of moth was the common variety. With the pollution resulting from industrialisation, the melanic form gradually became more common.

b Describe this process, making specific reference to the peppered moth example.

20 **a** Define evolution.

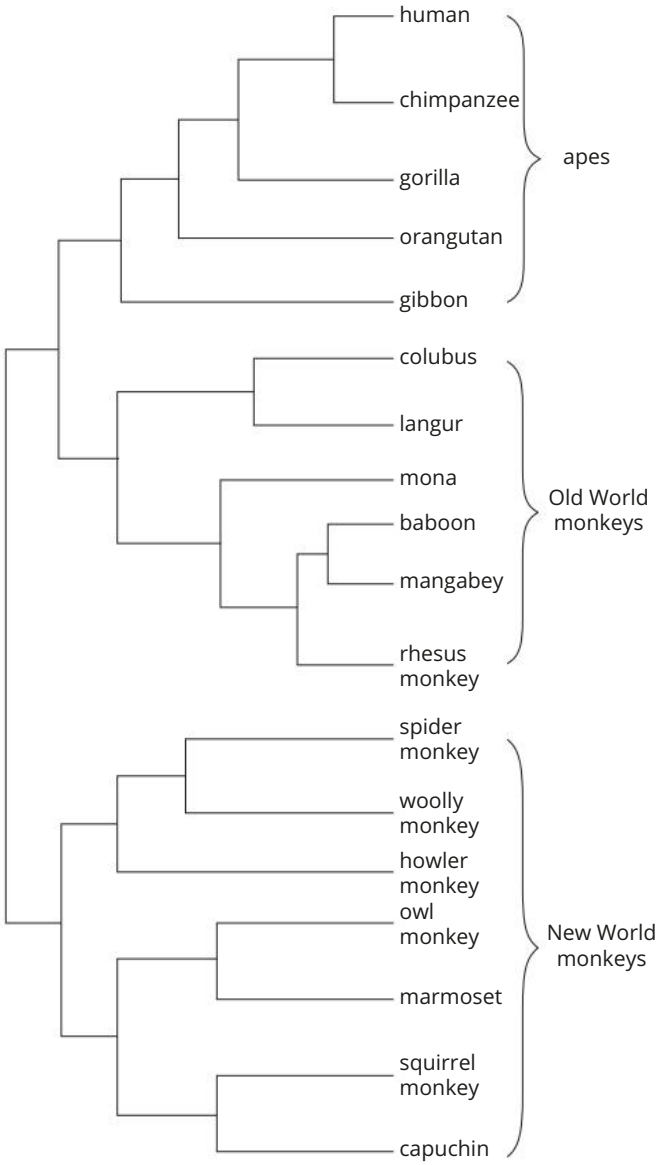
b Distinguish between the following terms.

i coevolution and parallel evolution

ii macroevolution and microevolution

21 State the three conditions required for natural selection to occur.

22 The proposed phylogenetic tree below for primates outlines three clades: apes, Old World monkeys and New World monkeys. All apes and listed Old World monkeys have trichromatic (three-colour) vision. Among the New World monkeys, only the howler is fully trichromatic; most are dichromatic and are able to see either red or green but not both. The owl monkey is unable to perceive colour (monochromatic).



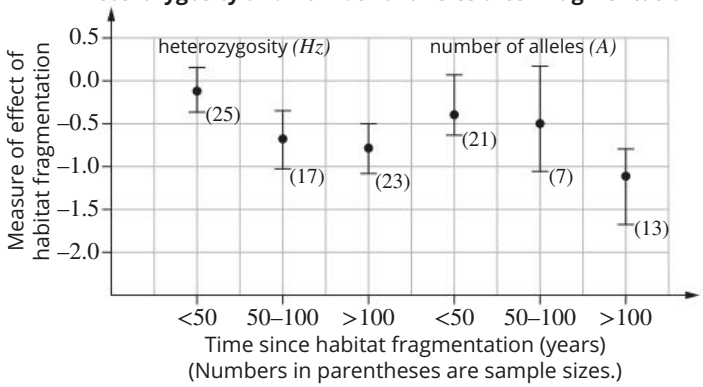
a Identify the position of the common ancestor of apes and Old World monkeys, by circling it on a copy of the phylogenetic tree.

b **i** It has been proposed that the evolution of trichromatic vision in howler monkeys is an example of convergence. Justify this proposal using the phylogenetic tree.

ii An alternative hypothesis is that the common ancestor of the New World monkeys had trichromatic vision. Discuss which theory is the more likely.

23 A 2008 study considered various other studies examining the effects of habitat fragmentation on genetic diversity. Fragmented sites were compared with unfragmented sites and the genetic diversity of plant populations was determined. The study areas were considered in three groups: areas fragmented for less than 50 years, areas that became fragmented between 50 and 100 years ago, and areas that underwent fragmentation more than 100 years ago. A statistical comparison was undertaken to determine the difference in genetic diversity between the fragmented sites and other similar sites that were undisturbed large areas. The results of this study are shown in the graph below.

Weighted means of the effect of habitat fragmentation on heterozygosity and number of alleles after fragmentation



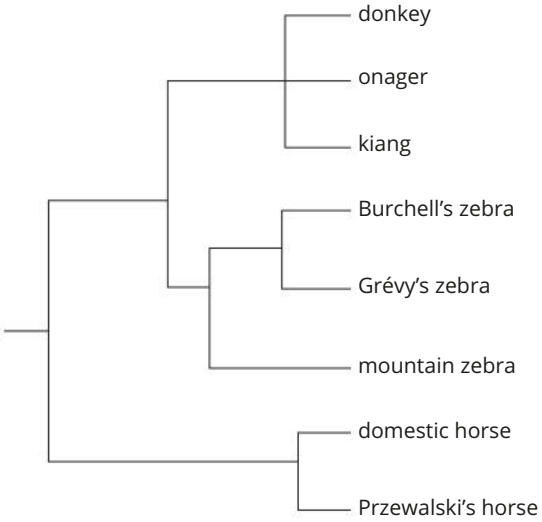
The analysis used a figure of 0.2 or less to indicate no significant difference between the sites, 0.5 to 0.8 as a moderate difference, and 0.8 or more indicating a large difference. The range bars indicate the 95% confidence intervals.

a **i** Conclude the effect of habitat fragmentation on genetic diversity.

ii Justify your conclusion.

b Propose the consequences for long-term population survival of the species surveyed in the study as a result of the effects of habitat fragmentation on their genetic diversity. Justify your proposal.

24 The genus *Equus* includes several species, both extinct and extant (still living). The extant species includes two species of horse: Przewalski's horse (*Equus przewalskii*) and the domestic horse (*Equus caballus*). Przewalski's horse is listed as endangered. In 1945, 13 individuals were held in captivity. All current species members are descendants of nine members of this captive population. The phylogenetic tree for the genus *Equus* is shown below.



a The population of Przewalski's horse from which the current population descended was quite small in number. Explain whether this represents a genetic bottleneck or a founder population.

b Despite the considerable increase in the numbers of Przewalski's horse, it is still considered to be endangered. Explain why this is the case.

c The nearest living relative of Przewalski's horse is the domestic horse. Przewalski's horses have 66 chromosomes and domestic horses have 64. Despite this difference, matings between domestic horses and Przewalski's horses produce fertile offspring.

i Propose how the change in chromosome numbers could have occurred.

ii Przewalski's horse and the modern domestic horse are very closely related. The relationship is so close that some taxonomists classify them as subspecies of the same species: *Equus ferus*. Define subspecies.

25 During a severe storm a small flock of birds is blown to an offshore island. The flock is now separated from the main population as the distance between the mainland and island is too far for them to return.

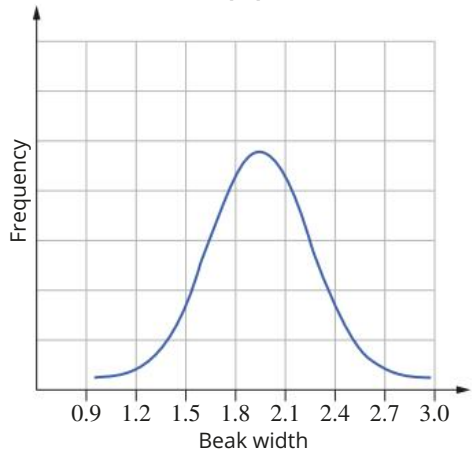
a i State the term used to describe the small flock of birds.

ii This situation frequently leads to allopatric speciation. Define allopatric speciation.

iii Explain why geographical isolation can lead to speciation.

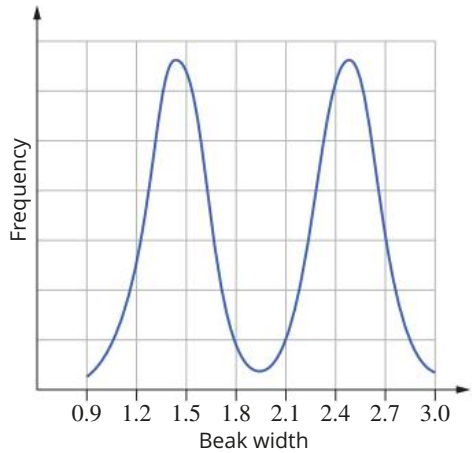
A group of evolutionary biologists studying the ecology of the island studied the bird population from its arrival on the island for 20 generations. Many measurements of the birds were taken. One such measurement was beak width. The graph below shows the range of widths of the beaks of the initial population.

Beak width and distribution of initial population



After 20 generations the distribution of beak widths is as shown in the graph below.

Distribution of beak widths over 20 generations



b Name the type of selection that has resulted in this change in distribution of beak sizes.

c After another 20 generations, the original population had split into two populations. Careful observation of the birds showed that both populations were seed eaters, one of which fed on small grass seeds in open fields while the other consumed much larger seeds produced by trees in forested areas.

i Explain how the separation into two populations could have occurred.

ii Over time this situation could lead to the formation of new species. Describe the processes through which this would occur.

iii Name the type of speciation involved.

26 The greater prairie chicken (*Tympanuchus cupido*) once had a range that covered most of the central states of the USA. Hunting and habitat loss have reduced their range to several isolated populations, some of which are on the verge of local extinction. Greater prairie chickens do not migrate and are highly territorial in their behaviour. They prefer to inhabit the open grasslands of the prairie but are sometimes found on cultivated agricultural land.

Their current distribution is shown on the map. Due to the large reduction in numbers and fragmentation of the once continuous population, the greater prairie chicken has been the subject of a number of studies and some human interventions in order to reduce the chance of extinction of some of the populations. One such study looked at average number of alleles per locus in four of the populations as shown in the table.

Distribution of the greater prairie chicken

Greater prairie chicken average alleles per locus in four populations

Location	Year of data collection	Population size	Number of alleles per locus
Illinois (IL)	1930–60	1000–25 000	5.2
	1993	less than 50	3.7
Kansas (KS)	1998	750 000	5.8
Nebraska (NE)	1998	75 000–200 000	5.8
Minnesota (MN)	1998	4000	5.3

a i Describe how genetic diversity changed in Illinois between 1930 and 1993.

ii Explain why these changes are of concern to conservationists.

b Gene flow is believed to continue between some of the populations but others are isolated. Explain using the data how such a conclusion has been drawn.

In an effort to protect the Illinois population from extinction by increasing its genetic diversity, between late 1992 and 1998 individuals from other populations were introduced into the Illinois population. Between 2010 and 2013, in order to assess the success of the introduction, DNA was analysed to determine whether genetic diversity had been increased. Data was collected before and after the introduction. The researchers measured both allelic richness (number of different alleles) and heterozygosity. Higher values for both of these indicate greater diversity. The scientists' findings are shown in the next table.

Genetic diversity of the greater prairie chicken pre-translocation and post-translocation

		Pre-translocation	Post-translocation
Number of individuals sampled		**32**	**18**
heterozygosity	mean heterozygosity	0.525	0.611
	standard error	0.09	0.08
	standard deviation	0.9266	0.5347
allelic richness	richness	4.7	5.5
	standard error	0.9	0.6
	standard deviation	1.0832	0.4628

c Evaluate the data and discuss whether the translocation has been successful in increasing the genetic diversity of the Illinois population of the greater prairie chicken.

Glossary

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5' cap A specialised guanine nucleotide at the 5' end of a messenger RNA molecule.

A

abiotic The non-living (physical and chemical) parts of the environment, such as soil, water and sunlight.

absolute uncertainty The uncertainty of a measurement that results from the smallest available increment on an instrument.

abundance The number of individuals in a population.

abyssal The deep ocean, especially that between 3000 and 6000 m below sea level.

accuracy The condition or quality of being true, correct or exact; freedom from error or defect; precision or exactness; correctness; in science, the extent to which a measurement result represents the quantity it purports to measure; an accurate measurement result includes an estimate of the true value and an estimate of the uncertainty.

adaptive radiation A single species evolving into several species by adapting to the requirements of different environments and lifestyles. This process is often quite rapid.

adenine (A) One of the five nitrogen-containing bases found in DNA and RNA. The others are cytosine, thymine, uracil and guanine.

adenosine triphosphate (ATP) A molecule that provides energy for immediate use by a cell; produced during glycolysis and cellular respiration.

algal bloom A sudden and exponential increase in the population of algae in a waterway, usually caused by an excess of nutrients in the water.

allele An alternative form of a gene.

allele frequency The relative proportion of a particular allele in a gene pool, typically presented as a decimal or percentage of the allele of that gene in the gene pool.

allelopathy A situation where an organism produces, and releases into the environment, chemicals that inhibit another organism's growth, survival or reproduction.

allopatric speciation The divergent evolution of two new species from an ancestral species resulting from separation by a geographicalal barrier. The lack of gene flow between populations and the slight variations in environmental conditions of the two habitats drives natural selection in slightly different ways until the two populations become genetically distinct.

allosome A chromosome that is involved in sex determination. In humans, the allosomes are the X and Y chromosomes. Also called a sex chromosome.

amensalism A relationship between organisms of different species in which one of the organisms benefits and the other is harmed or killed.

ammonification The process where bacteria decompose dead organic remains and convert them into ammonia.

anaphase The third phase of mitosis where the spindle fibres contract, pulling the two chromatids apart.

aneuploidy A condition in which an individual does not have the correct diploid number of chromosomes. As a result of non-disjunction of homologous chromosomes during meiosis, individuals typically have an extra copy (trisomy) or only one copy of a particular chromosome.

anneal The joining together of DNA or RNA fragments by complementary base pairing.

anomaly Something that deviates from what is standard, normal, or expected (Taylor 1982).

anoxic Lacking in oxygen.

anticodon The three bases in the tRNA molecule that are complementary to the triplet codon in the mRNA.

antiparallel A term describing the two strands in DNA. One runs 5' to 3', the other runs 3' to 5'.

apoptosis The death of cells that occurs as a normal and controlled part of an organism's growth or development.

aquifer An underground water supply found in areas of porous rock.

artificial selection The process of changing the allele frequencies of a population through human intervention; a form of selective breeding.

atmosphere The gaseous envelope surrounding the Earth; the air.

autism A complex developmental disability that impairs a person's ability to communicate and interact with others.

autosome A chromosome that is not a sex chromosome.

autotroph An organism that is able to produce its own food from inorganic materials using light (photoautotroph) or chemical (chemoautotroph) energy. All autotrophs are producers.

B

background extinction The average rate of natural loss of species.

bacteria The prokaryotes that do not contain chlorophyll and whose cell walls contain a layer of peptidoglycan. They do not possess any membrane-bound organelles.

bacterial transformation The incorporation of DNA from another organism into a bacterial cell.

bacteriophage A virus that infects bacteria.

bar graph A graph that shows the measured value (or value of central tendency) of the dependent variable by the length of the horizontal bar.

basal cover A measurement of the cross-sectional area of the stem or stems of plants to be surveyed at soil level.

base One of the five nitrogenous chemicals present in the nucleotides of nucleic acids (DNA or RNA). The five bases are adenine, guanine, cytosine, thymine (DNA only) and uracil (RNA only).

Basic Local Alignment Search Tool (BLAST) Software that has an algorithm for finding and comparing biological sequence information, such as the amino acid sequences of proteins or the nucleotides of DNA sequences.

belt transect A wide transect line that gives information on the abundance as well as the presence of different species.

benthic region The region at the bottom of a body of water, such as a lake or river bottom or the sea floor.

binary fission A form of asexual reproduction in unicellular organisms in which the parent cell divides into two approximately equal parts.

binomial A two-part Latin or Greek name of a species of living organism consisting of genus and species.

binomial system The method of classification in taxonomy devised by Carl Linnaeus, where every species is designated a two-part name.

biodiversity Biological diversity; the variety of all life forms, the genes that they contain and the ecosystems of which they are a part.

biofilm A thin, sticky layer of bacteria and other microbes on a surface.

biogeochemical cycle The cycling of elements and compounds through the living and non-living parts of the Earth.

bioinformatics The science of collecting and analysing complex biological data, such as genetic codes.

biological classification The process by which scientists group living organisms.

biological fitness An organism's ability to survive and reproduce in its natural environment.

biological species A group of naturally interbreeding populations that are reproductively isolated from other groups (or species) of organisms.

biomass The total quantity of biological matter of a group of organisms in a given area. Biomass consists of the carbohydrates, lipids, proteins and other organic molecules that comprise the group of organisms. It is measured as matter per unit area (e.g. kg m^{-2}) or the equivalent amount of energy bound in the mass of tissue (e.g. kJ m^{-2}).

biome A group of communities that have similar structures and habitats and that extend over a large area (e.g. a coral reef).

bioregion Large, geographicalally distinct areas of land with common characteristics such as geology, landform patterns, climate, ecological features and plant and animal communities.

biosphere The region of the Earth's land, sea and air that is occupied by living things.

biotic Relating to the biological (living) parts of the environment.

bioweapon A harmful biological agent used as a weapon of war.

block mutation A mutation that affects a large section of a chromosome.

blunt-end restriction enzyme A restriction enzyme that leaves clean-cut ends because it cuts both strands of the DNA molecule at the same location within the recognition site.

bottleneck effect The impact of a large portion of a population being removed from a habitat by chance, typically as a result of a natural disaster.

brackish Water that is neither fully saline (as sea water) or fully fresh.

branch An internode, or section on a phylogenetic tree connecting two nodes.

C

calibration The adjustment of an instrument with a standard scale of readings.

canopy cover A measure of the total area covered by a plant, including its canopy, best seen by the finding the edge of the shadow cast by the canopy of the plant when the sun is directly above.

carbon fixation The incorporation of carbon into organic compounds by living organisms.

carcinogen A substance that damages cell DNA. A carcinogen can be physical, chemical or biological.

carnivore An organism that feeds only on other animals.

carrier (1) An organism infected by a pathogen, usually without being affected by the pathogen, that can transmit the pathogen to another organism. (2) An organism with an allele for a genetic disease, but that does not show the disease because it is heterozygous and the other allele is expressed.

carrying capacity In Biology, the size of population that can be supported indefinitely on the available resources and services of that ecosystem.

Cas protein A protein associated with CRISPR.

cell cycle The events in the life of a cell, from its formation by cell division through its growth and function until it divides again.

cell plate A partition that forms in a plant cell during cell division. It divides the cell into two parts, each of which develops into a daughter cell.

cell replication The process by which a single cell divides into two or more daughter cells.

centriole A small cylindrical organelle consisting of a group of microtubules, and occurring as a pair in the centrosome in the cells of animals and some other organisms.

centromere The point on a chromosome at which chromatids are held together and to which the spindle fibres attach during mitosis. It is visible during cell division as a constriction along the length of a contracted chromosome.

chemical (HAZCHEM) codes A system of codes and images that provides warnings of hazards about items to users.

chemoautotroph An organism that can synthesise organic compounds from inorganic compounds using the energy released by simple chemical reactions. Also called a chemosynthetic autotroph.

chemosynthesis The process of carbon fixation using energy from inorganic chemical reactions.

chemosynthetic autotroph An organism that can synthesise organic compounds from inorganic compounds using the energy released by simple chemical reactions. Also called a chemoautotroph.

chiasma A point of crossing over of strands of non-sister chromatids observed during the first division of meiosis.

chromatid One of two identical chromosomal strands into which a chromosome splits before cell division.

chromatin A complex structure of DNA and proteins found in the nucleus of eukaryotic cells.

chromosome A thread-like structure in the nucleus, composed of DNA and proteins. Contains genetic information in the form of genes arranged in a linear order. Found in constant numbers in body cells of organisms from a particular species.

clade A group of organisms that consists of a common ancestor and all its lineal descendants.

cladistics A biological classification system based on phylogenetic relationships.

cladogram A taxonomic diagram of inferred relationships within a clade.

claim An assertion made without any accompanying evidence to support it.

class A group in the hierarchy of groups and sub-groups of living organisms. It is smaller than a phylum and larger than an order.

classification The categorising of living organisms into taxonomic groups.

climax community The final community in a succession, which remains stable for an indefinite period of time.

co-dominance The occurrence of a phenotype in a heterozygote that results from the expression of both alleles. An example is the AB blood group in humans.

coding DNA A sequence of DNA that codes for a protein.

coding strand A coding strand of DNA whose base sequence is used as the template for the formation of an mRNA strand.

codon The basic unit of the genetic code; a sequence of three bases on a mRNA strand that specifies a single amino acid or directs translation to begin or to stop.

coenocyte A large multinucleate cell that results from repeated mitosis that is not followed by cytokinesis.

coevolution The evolution of two species in response to one another, such as a pollinator and flowering plant or a parasite and host.

column graph A graph that shows the measured value (or value of central tendency) of the dependent variable by the height of the column.

commensalism A relationship between two organisms in which only one organism benefits, but the other organism is not harmed.

common ancestor An organism from which two or more species diverge. Also known as a shared ancestor.

community A group of species that occur in the same area and interact, or could interact, with each other.

comparative genomics The comparison of genomic features of different organisms, including comparing whole genomes, genes, regulatory sequences and genome organisation.

competence The ability of a cell to alter its genetics by taking up extracellular DNA from its environment.

competition The interaction between organisms that are seeking to use the same resource, such as food water, shelter, sunlight or mates.

competitive exclusion The principle that says that two species that have the same requirements and live in the same area will compete to the point at which the less fit species becomes extinct in that ecosystem. Also known as Gause's Law.

complementary base pairing The pairing between adenine and thymine, and guanine and cytosine, in a DNA molecule.

complementary DNA (cDNA) Double-stranded DNA that contains no introns; copied from mRNA by the enzyme reverse transcriptase.

condensation polymerisation A reaction in which two molecules become covalently bonded to each other through the loss of a small molecule, usually water.

confidence interval A calculated range in values that estimates where the true value is likely to be, according to a pre-determined level of confidence.

constitutive gene A gene that is continually being expressed in cells.

consumer An organism that obtains the organic compounds it needs by consuming other organisms or their products.

continuous variation Variation within a population that is smoothly graded.

control group A selected test group (sample group) in which no change to the independent variable exists, which represents typical natural conditions.

controlled variable A variable that is controlled during an experiment so that it does not change and does not influence the measured results or dependent variable.

convergent evolution The evolution of similar features in unrelated groups of organisms (i.e. that do not share a recent common ancestor).

CRISPR A gene editing technique derived from the bacterial immune system.

cross The intentional breeding of two genetically different organisms that results in offspring that inherit genetic material from each parent.

crossing over The exchange of chromosomal material between non-sister chromatids of a homologous chromosome pair during prophase I of meiosis.

cystic fibrosis A genetic disease in which the exocrine function of cells are affected due to changes in the protein structure of the chloride channels in cell membranes. It mainly impairs function of the lungs and digestive system of patients.

cytokinesis The division of a cell following mitosis or meiosis, when the cytoplasm divides and the cell splits into two daughter cells.

cytosine (C) One of the five nitrogen-containing bases found in DNA and RNA. The others are adenine, thymine, uracil and guanine.

D

Darwinian theory The theory of biological evolution by natural selection developed by Charles Darwin.

decomposer An organism that breaks down dead or decaying organisms or organic wastes by producing enzymes that they secrete into the environment. Decomposers are mostly bacteria or fungi.

degenerate A genetic code in which several of the codons code for the same amino acid.

deletion mutation (1) A type of point mutation that involves the loss of one or two nucleotides in a sequence and causes a frameshift mutation. (2) A type of block mutation in which multiple genes are cut from a chromosome.

denitrification The process of turning nitrate into nitrogen gas.

denitrifying bacteria Bacteria that process nitrates in the soil and release the nitrogen back into the atmosphere.

density-dependent factor A limiting factor on population size whose effect depends on the size of the population.

density-independent factor A limiting factor on population size whose effect does not depend on the size of the population.

deoxyribonucleic acid (DNA) A nucleic acid made up of a sequence of deoxyribose sugars and bases (adenine, cytosine, guanine and thymine) linked by phosphate bonds. It is the carrier of genetic information in all cellular organisms and most viruses, and is found in chromosomes (and mitochondria and chloroplasts).

deoxyribose The five carbon sugar found in DNA.

dependent variable The variable that is measured or observed during an experiment; assumed to change due to changes in the independent variable.

detritivore An organism that obtains its nutrients by consuming decomposing organic material.

detritus Organic matter consisting of the dead remains of organisms or wastes expelled by animals.

deviation The extent to which a set of data differs from the mean. See also standard deviation.

dichotomous key A key that has two branches at each step and is used to identify taxonomic groups.

dideoxynucleotide (ddNTP) A chain-elongating nucleotide triphosphate that inhibits DNA polymerase.

dihybrid cross A cross between pure lineages that exhibit two different phenotypes.

diploid (1) The state of having two sets of chromosomes ($2n$). All somatic cells are diploid. (2) A cell that contains the full set of chromosome pairs, as in body cells.

directional selection Natural selection that favours a particular phenotype and skews allele frequency in one direction.

discrete variation Variation within a population that is segmented in two or more groups.

disease Any condition that impairs the normal functioning of an organism.

disruptive selection Natural selection that favours extreme phenotypes and skews allele frequency towards both ends of the distribution.

distribution The geographical extent of a group of organisms.

divergent evolution The evolution of two or more different species from a common ancestral species.

DNA amplification The process of creating millions of identical copies of a DNA sample using the polymerase chain reaction.

DNA helicase An enzyme that drives the separation of the DNA at the point of the replication fork, during DNA replication.

DNA hybridisation A molecular biology technique used to measure the degree of genetic similarity between two sections of DNA.

DNA ladder A set of DNA molecules of known size used as a 'molecular ruler' on gel electrophoresis to determine the size of other DNA molecules.

DNA ligase An enzyme that joins together fragments of DNA by forming a phosphodiester bond between the 3'-hydroxyl and 5'-phosphate of adjacent nucleotides.

DNA polymerase An enzyme that catalyses the elongation of a new DNA strand in DNA replication, using an existing strand of DNA as a template.

DNA profiling A technique that produces an individual's unique pattern of DNA bands on a gel by analysing short tandem repeat regions of the genome.

DNA replication The process in which a DNA molecule is copied to produce two identical DNA molecules.

DNA sequencing The determination of the sequence of bases in a fragment of DNA. DNA sequencing can be used to determine relationships between individuals of a species, and for determining the entire genome of an organism.

DNA thermocycler The machine used in the polymerase chain reaction that alters the temperature in pre-programmed steps.

domain The highest taxonomic ranking of living organisms.

dominance The expression of one allele of a gene rather than another allele of the same gene.

dominant phenotype The phenotype expressed in a heterozygous individual (an individual carrying different alleles of the same gene).

double helix The form of DNA; refers to the two adjacent polynucleotide strands wound into a spiral shape.

duplication mutation A block mutation that involves the repetition of a section of a chromosome, usually containing multiple genes. The duplication may involve thousands of repeats, significantly lengthening the chromosome.

E

ecological niche The role and space an organism fills in an ecosystem, including all its interactions with the biotic and abiotic factors of its environment.

ecological pyramid of energy A pyramid-shaped representation of the energy distributed at different trophic levels in an ecosystem. Producers are at the bottom and are the largest layer, and top-level consumers are at the top.

ecoregion A large region of the Earth's surface distinguished by its latitude and geography, and generally containing similar ecosystems.

ecosystem A system formed by living organisms interacting with one another and with their physical environment.

ecosystem diversity The variation in ecosystems in a particular region or across the world.

ectoparasite A parasitic organism that lives and feeds on the outside of a host, such as fleas and ticks.

ectothermic An organism whose body temperature varies with the environmental temperature.

electroporation A pulse of electricity used to open the pores of a cell to enable genetic material to be inserted.

emigration The movement of individuals out of a population to some other place.

endoparasite A parasitic organism that lives and feeds inside a host.

environment The sum total of all of the factors that affect an organism during its lifetime.

eon One of several subdivisions of geological time enabling cross-referencing of rocks and geological events from place to place. Eons are the largest subdivisions.

epigenetic inheritance The inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence.

epigenetics Any mechanism that alters gene expression without altering the DNA sequence.

epoch One of several subdivisions of geological time enabling cross-referencing of rocks and geological events from place to place. Epochs are the smallest subdivisions.

equilibrium A state of balance; a situation where the ecosystem is stable.

era One of several subdivisions of geological time enabling cross-referencing of rocks and geological events from place to place. Eons are larger subdivisions than eras; eras may be divided into periods and epochs.

error A measure of the estimated difference between the observed or calculated value of a quantity and its true value.

ethics The moral principles that govern a person's behaviour or how an activity is conducted.

eukaryote An organism composed of one or more cells that contain distinct membrane-bound nuclei and many organelles; eukaryotes include protists, fungi, plants and animals.

eutrophication A situation where the nutrients build up in a waterway, leading to it becoming very productive.

evolution Change in the genetic composition of a population during successive generations, which may result in the development of new species.

evolutionary radiation The rapid expansion and spread of a large number of related species from a single common ancestor. Also known as adaptive radiation.

exon A coding region of a eukaryotic gene that is expressed. Exons are separated from each other by introns.

experimental group The selected test group (sample group) in which the independent variable has changed to elicit a change in the measured results.

exponential growth A growth rate that is always increasing. When graphed, it produces a J-shaped curve.

extend In science, to extend an experiment is to modify the methodology to overcome limitations of the scope or applicability of the data.

extinction The dying out of a species; when the individuals of a species no longer exist.

extraneous variable Any other variable, besides the independent and dependent variables, that influence results.

F

F1 generation The generation consisting of offspring of a cross between members of the parental generation.

F2 generation The offspring of a cross between members of the F1 generation.

family A group in the hierarchy of groups and sub-groups of living organisms. It is smaller than an order and larger than a genus.

fecundity The reproductive rate of an organism, measured by the number of offspring produced.

fertilisation The penetration of an egg by a sperm, and fusion of the egg and sperm nuclei.

fixed When allele frequency at a gene locus in a gene pool changes from having more than one allele at the locus to having only one allele at the locus.

food chain A sequence of feeding relationships, beginning with a producer and ending with a higher order consumer. The producer is eaten by a first-order consumer, the first-order consumer is eaten by a second-order consumer, and so on.

food web A network of interlinked food chains that describes the feeding relationships between all organisms in an ecosystem.

form The shape, structure or appearance of an entire living organism (or part thereof).

fossil The preserved remains, impressions or traces of an organism found in rocks, amber (fossilised tree sap), ice or soil.

fossil record The record of the evolution of organisms through geologicalal time based on information from fossils.

founder effect When a small portion of a population disperses to a new location and becomes genetically isolated from the main population. The allele frequencies of the founding population are completely dependent on those of the specific individuals that were relocated, and therefore may be significantly different from those of the original population.

frameshift mutation A mutation occurring when the number of nucleotides inserted or deleted is not a multiple of three, resulting in incorrect grouping into codons.

fundamental niche The potential niche that a species would occupy if there were no competition from any other species.

G

gamete A haploid cell capable of fusion with another haploid cell to form a zygote. In vertebrates, the gametes are sperm and egg cells.

gel electrophoresis A technique for separating fragments of DNA, or different proteins, based on their molecular weight (or length). Fragments migrate through a gel at rates that are dependent on their length and charge

gene Region/s of DNA that are made up of nucleotides; the molecular unit of heredity.

gene cloning The production of identical copies of a gene.

gene editing The modification of genes by removal, substitution or alteration by mutation, without necessarily introducing a foreign gene.

gene expression The process by which the information stored in a gene is used to synthesise a functional gene product (protein or RNA).

gene flow The movement of alleles between individuals of different populations; includes the dispersal of pollen and seeds in plants.

gene mapping The determination of the location of genes, and the distance between them, on a chromosome.

gene pool All the alleles possessed by members of a population that may potentially be passed to the next generation.

gene probe A section of DNA with a base sequence complementary to a particular gene, to which it base pairs. When labelled with a fluorescent dye or radioactive marker, it is used to find a particular DNA sequence within a DNA fragment.

gene regulation A biological process that leads to a gene being transcribed or not transcribed in particular cell types or stages of development.

gene therapy The replacement of faulty genes by genetic engineering techniques.

genetic code The particular arrangement of nucleotides in the DNA molecule.

genetic diversity The variation in the genetic constitution of a community of species and all of the individuals of all of the species within that community.

genetic drift A random change to allele frequencies in a gene pool as the result of a chance event. Genetic drift has a more significant impact on smaller populations, as the chance death of one individual could eliminate an allele from the gene pool.

genetic isolation The prevention of gene flow between two populations.

genetic modification The transfer of genes from one organism to another.

genetically modified organism (GMO) An organism that has had its genome altered.

genome All the genetic material in the chromosomes of an organism, including its genes and DNA sequences.

genotype The genetic composition of an individual.

genus A group in the hierarchy of groups and sub-groups of living organisms. It is smaller than a family and larger than a species.

geographicalal isolation The process of separation of populations by physical and geographicalal barriers.

geologicalal timescale The timescale of events that have occurred on Earth from its formation to the present time.

germ cell A cell in an organism that gives rise to gametes.

germline A cell that can continue through successive generations of an organism.

germline mutation A genetic mutation that occurs in the genetic material of an egg or sperm and can therefore be inherited by future generations.

Gondwana A supercontinent of the past that formed when the process of plate tectonics united the land masses of the southern hemisphere; includes present-day Africa, Madagascar, India, Australia, Antarctica and South America.

gradual speciation See speciation.

gross primary productivity The amount of production from primary producers created in a given time. It can be measured as accumulation of energy or accumulation of biomass.

ground cover The area of the soil covered by plants, plant litter, rocks or any other material.

guanine (G) One of the five nitrogen-containing bases found in DNA and RNA. The others are cytosine, thymine, uracil and adenine.

guide RNA (gRNA) A short, synthetic RNA composed of a scaffold sequence necessary for Cas 9 binding.

H

habitat The environment where an organism or species lives, grows and reproduces.

habitat fragmentation The process of separation of populations when a habitat is divided into smaller segments, e.g. by natural geologicalal activity or human activity.

haemoglobin A red, respiratory pigment, found in many animals, that increases the oxygen-carrying capacity of blood.

haploid (1) The state of having one set of chromosomes (n). Gametes are typically haploid so that fertilisation restores the diploid number of the organism. (2) A cell that contains only half the normal number of chromosomes (one of each pair).

hemizygote A diploid cell or organism with only one copy of a particular chromosome. Human males are hemizygotes because they have one X chromosome rather than two.

herbivore An animal that feeds only on producers, such as plants or algae.

heterogametic The state of having different sex chromosomes, e.g. human males (XY).

heterotroph An organism that must obtain nutrients by consuming other organisms.

heterozygote A diploid individual with different alleles for a particular gene.

hierarchy A series of ordered groupings from most to least diverse in a taxonomic system of classification.

histone A small protein that binds to DNA and plays a key role in its chromatin structure.

homeotic gene A gene that controls the overall body plan of animals by controlling the developmental fate of groups of cells

homogametic The state of having two similar sex chromosomes, e.g. human females (XX).

homologous chromosome A matching pair of chromosomes in a diploid organism. Homologous chromosomes carry the same genes in the same loci.

homozygote A diploid individual with two identical alleles at a particular genetic locus.

horizon A layer in a soil profile.

host The organism upon which a parasite lives and feeds.

***Hox* gene** A master regulatory gene that affects the expression of other genes to control the genes that determine the body plan of animals.

Human Genome Project An international scientific research project to determine the sequence of nucleotide base pairs that make up human DNA.

human microbiome The collective genomes of all the microbes that live inside and outside the human body.

humus Decaying organic matter found in the upper layer or layers of the soil.

hybrid An individual produced by a cross between parents with different genotypes.

hybrid inviability A form of postzygotic isolation between different species where any fertilised hybrid zygotes do not develop properly and do not reach birth.

hybrid sterility A form of postzygotic isolation between different species where hybrid offspring that survive to reproductive maturity are incapable of reproducing.

hydrosphere The watery region of the Earth, consisting of the oceans, seas, lakes, streams and groundwater.

hypothesis In science, a tentative explanation for an observed phenomenon, expressed as a precise and unambiguous statement that can be supported or refuted by experiment (ACARA 2015c).

hypoxic An environment with an oxygen level between 0 and 2 mg L^{-1}.

I

immigration The movement of members of a species into a population from elsewhere.

incomplete dominance A form of dominance where a phenotype is not completely dominant, causing intermediate phenotypes to occur. The presence of intermediate phenotypes is because one allele for a specific trait is not completely expressed over another allele.

independent variable The variable during an experiment that is deliberately altered, or selected to be tested, which is assumed to cause a change in the dependent variable.

induce To promote or activate.

induced mutation A mutation that occurs following exposure to mutagens.

inducible operon A unit of DNA regulated by other promoter molecules, which can be switched on or off.

infectious Able to be passed from one organism to another.

ingroup In cladistics analysis, the groups of organisms that are more closely related to one another than they are to an outgroup on the same tree.

insertion mutation (1) A point mutation that involves the addition of one or two nucleotides into the existing sequence. (2) A block mutation that occurs as a result of one part of a chromosome breaking off and attaching to another chromosome.

interphase The phase in the cell cycle when the cell is not undergoing mitosis.

interspecific competition Competition between members of different species.

interspecific hybrid An individual produced by a cross between parents with different genotypes; often used to refer to offspring of different species.

intertidal zone A coastal zone that is only exposed during parts of a 24-hour period due to the action of tides.

intraspecific competition Competition between members of the same species, such as competition for mates or nesting sites.

intron A noncoding intervening sequence of DNA within a eukaryotic gene.

inversion mutation A type of block mutation that involves the reversal of a sequence on a chromosome.

K

***K*-selection** Where species in stable environments produce low numbers of slow-developing offspring.

***K*-strategist** Organisms that practise *K*-selection.

karyotype A description of the number, size and shape of chromosomes observed in an individual.

keystone species A plant or animal that plays a unique and crucial role in the way an ecosystem functions.

kingdom A group in the hierarchy of groups and sub-groups of living organisms. It is smaller than a domain and larger than a phylum.

L

***lac* operon** A segment of DNA in *Escherichia coli* that codes for three enzymes that break down lactose into glucose and galactose. It is only switched on in the presence of lactose in the environment.

***lac* repressor** A region of DNA adjacent to the *lac* operon that inhibits the expression of the *lac* operon until there is lactose available.

lacI A regulatory gene that codes for the *lac* repressor.

lactose A disaccharide sugar composed of two monomer sugar units: glucose and galactose.

***lacZ* gene** A gene in the *lac* operon that codes for beta galactosidase; used in recombinant plasmids for detecting transformed bacteria.

lagging strand The complement of the leading strand that faces in the opposite direction to the movement of the replication fork. Replication on the lagging strand is discontinuous and results in small segments called Okazaki fragments.

Law of Independent Assortment The principle, first stated by Gregor Mendel, that individual inherited traits assort independently, so that the occurrence of a trait (such as brown eyes) in an offspring is independent of the occurrence of any other trait (such as attached ear lobes). Because of linkage, the law applies only to alleles on different chromosomes. Also called Mendel's second law of inheritance.

leaching The loss of nutrients from the soil as they dissolve in rain water and are removed from the site.

leading strand The DNA strand that is replicated in the same direction as the replication fork is moving. Its synthesis occurs more easily and quickly than the lagging strand.

leaf An individual species on a phylogenetic tree.

leaf cover A measure of canopy coverage. It can be determined by the area of shadow cast by the canopy when the sun is directly above the sample site. It does not include areas in the canopy that do not contain leaves.

ligase An enzyme that joins together two molecules or fragments of molecules.

ligation The process of joining two fragments of DNA using a DNA ligase enzyme.

limiting factor Any factor that prevents a population from growing larger. Common limiting factors are the availability of water, food, shelter, nesting sites and mates.

Lincoln index A formula and process for estimating population size.

line graph A graph that displays continuous data using a line from one point of measurement to the next.

line transect A transect line that gives information on the presence of different species.

lineage A series of organisms that are connected by a continuous line of descent from ancestor to descendant.

linkage The tendency for two or more genes on the same chromosome to be inherited together because they are close together on the chromosome. Linked genes may be separated if crossing over occurs between them.

Linnaean system The system of taxonomic classification and binomial nomenclature devised by Carl Linnaeus.

Linnaeus A Swedish botanist, Carl Linnaeus, who devised the current binomial system for classifying living organisms.

lithosphere The solid outer layers of the Earth, including the crust and the solid part of the mantle below.

littoral zone The area from the high water mark of a body of water to a depth beyond which insufficient light penetrates to support photosynthetic organisms

locus A particular place along the length of a chromosome where a given gene is located.

logistic growth A pattern of population growth where the growth slows as the size of the population nears the carrying capacity and then stabilises at the carrying capacity of the ecosystem.

lyse To burst open.

M

macroevolution The variation of allele frequencies at or above the level of species over geologicalal time, resulting in the divergence of taxonomic groups, in which the descendant is in a different taxonomic group to the ancestor.

macromolecule A molecule containing a very large number of atoms, such as proteins, nucleic acids or synthetic polymers.

mass extinction The large-scale worldwide extinctions evident in the fossil record and caused by major disruptive changes to global climate and the shifting of continents.

massively parallel sequencing The high speed, simultaneous analysis of DNA sequences.

maximum parsimony The principle of parsimony is applied when creating phylogenetic trees and the most closely related organisms are those that have the fewest changes in character. Maximum parsimony is the optimal criteria where all minimal states have been used in the tree construction.

mean A calculated value that represents the centre of a set of numbers that have been averaged.

measure of central tendency A value that estimates the central position in a set of data.

measured variable A variable that is measured during an experiment because it cannot be controlled, and must be known so that any influence on the measured results or dependent variable can be determined.

median The value in the middle of an ordered list of values.

meiosis A division of a nucleus that results in one copy of each homologous chromosome and one sex chromosome in each daughter cell. Meiosis produces four genetically unique daughter cells, each with half the number of chromosomes of the parent cell.

meiosis I The first round of division in meiosis during which homologous chromosomes are separated, reducing the chromosome number by half (reduction division) and producing two haploid daughter cells.

meiosis II The second round of divisions in meiosis during which the sister chromatids are separated to produce four haploid daughter cells.

mesopredator A predator that occupies a position in the middle of a food chain.

messenger RNA (mRNA) An RNA molecule transcribed from DNA.

metaphase The second phase of mitosis, where chromosomes align in the middle of the cell.

microevolution Small-scale variation of allele frequencies within a species or population, in which the descendant is of the same taxonomic group as the ancestor.

microhabitat A small living space within a larger habitat.

microinjection The injection of a liquid substance at a microscopic level.

microsatellite A short repeated sequence of nucleotides found at a defined locus on a chromosome. The number of repeats varies between individuals, so these are useful in DNA profiling.

migration The movement of individuals either into (immigration) or out of (emigration) a population of a species living at one place at one time.

mitochondrial DNA (mtDNA) The double-stranded circle of DNA found in the mitochondria of eukaryotic cells.

mitosis The division of a nucleus that results in two cells that are genetically identical to the parent cell. Asexual reproduction and cell replication for growth occur by mitosis.

mode The value that occurs most often in a list of values.

modify Change the form or qualities of; make partial or minor changes to something.

molecular homology A DNA or protein molecule (or their precise sequence) shared by two species or other taxa because of common ancestry.

molecular method The manipulation and analysis of DNA, RNA and protein.

monogenic A phenotype (characteristic) determined by a single set of genes.

monohybrid cross A cross between individuals that have different pairs of alleles of a particular gene. Monohybrid crosses are used to study the inheritance of one characteristic.

monomer A smaller subunit of a larger unit (called a polymer), e.g. amino acids and nucleotides.

monophyletic group A clade where all descendants come from the same ancestor, e.g. the Galapagos finches.

morphological The size, shape and appearance of biological structures.

mortality The death rate in a population, usually expressed as number of deaths per unit population in a given time period.

multi-access key An identification key that has a database of characters and states that allows the user to select any character rather than go through the systematic progression of a dichotomous key.

mutagen A chemical or physical agent that interacts with DNA and causes a mutation.

mutation A rare and permanent change in the DNA of genes that ultimately creates genetic diversity. A mutation can have a beneficial effect, a harmful effect or no effect at all on the survival ability of an individual.

mutation rate The change in a taxon's DNA over time, expressed as the number of nucleotide mutations that occur every million years.

mutualism A symbiotic relationship between two organisms in which both organisms benefit. An example is the pollination of flowers by insects, in which the insect receives nutrition and the plant is able to reproduce.

N

nanopore sequencing Direct DNA sequencing technique.

natality The birth rate in a population, usually expressed as number of births per unit population in a given time period.

natural selection A mechanism of evolution. Phenotypes with high adaptive values are 'selected for' because they are better suited to the environmental conditions. Individuals who are better adapted are more likely to survive and reproduce successfully and so are more likely to pass their alleles with high adaptive values on to the next generation.

negative selection A natural selection process that disfavours an allele or heritable trait.

net primary productivity The accumulation of biomass or energy by producers. It is the amount of energy or biomass left over after the producers have used some of the products of photosynthesis for metabolism.

network A diagram consisting of intersecting lines that show organism interactions within an ecosystem. It may include information about the type of interaction and/or the strength of the interaction.

niche The role of an organism in an ecosystem, often defined by the environmental, biological and other conditions in which it lives. A particular niche, such as a predatory bird, may be filled by different animals in different ecosystems (e.g. the wedge-tailed eagle in Australia and the bald eagle in North America).

niche partitioning The process in which competing species become specialised in different ways in order to co-exist. Natural selection drives the process. Also known as niche segregation.

nitirification The process by which bacteria convert ammonia into nitrates.

nitrifying bacteria Soil bacteria that either convert ammonia or ammonium into nitrites, or nitrite into nitrates, thus enriching the soil with nitrogen-containing compounds which are usable by plants.

nitrogen-fixing bacteria Bacteria that convert atmospheric nitrogen into nitrogenous compounds, like nitrates, which can be used by plants.

nitrogenous base A nitrogen-containing molecule that is attached to the ribose sugar of DNA or RNA to form a nucleotide. There are five types of nitrogenous bases found in DNA and RNA: adenine, cytosine, guanine, thymine and uracil.

node The place of branching from a common ancestor to its descendants on a phylogenetic tree.

non-disjunction The failure of homologous pairs of chromosomes to separate during metaphase I of meiosis. Non-disjunction results in aneuploidy because two of the gametes formed will have two copies of the chromosome, while the other two gametes will be missing that chromosome entirely.

non-infectious Cannot be passed from one organism to another.

non-synonymous substitution mutation A mutation that results in a change in the amino acid sequence of a protein.

noncoding DNA A sequence of DNA that does not code for proteins, but may still be involved in the regulation of gene expression.

nonsense mutation A mutation that results in the generation of a 'stop' codon in a DNA sequence. These mutations generally have severe effects as they result in a shorter and usually non-functional protein.

nucleosome A particle made up of histone proteins around which DNA is coiled. Nucleosomes occur in chromosomes.

nucleotide A monomer, or building block, of the nucleic acids DNA and RNA, consisting of a phosphate, a sugar and a nitrogenous base.

null hypothesis The hypothesis that there is no relationship between two variables, so that any change that occurs in one variable when the other variable is changed is entirely random.

O

Okazaki fragment A discontinuous segment of DNA formed during replication of the lagging strand. Named after Dr Reiji Okazaki.

omnivore An organism that feeds on both plants and animals.

oncogene A gene that induces uncontrolled cell division leading to the development of neoplasms.

oocyte A precursor egg cell in the ovary that undergoes meiosis, resulting in the formation of a single egg cell.

oogenesis The process by which a female gamete (ovum) is produced.

operator A segment of DNA that binds to a transcription factor to regulate the expression of a gene.

operon A unit of genetic function common in bacteria consisting of co-ordinately regulated clusters of genes with related functions.

order A group in the hierarchy of groups and sub-groups of living organisms. It is smaller than a class and larger than a family.

origin In prokaryotes, the point at which the chromosome may be attached to the plasma membrane.

outgroup A taxon outside the area of interest of a clade, often used as a reference point for the ingroup within the clade.

outlier A value that 'lies outside' (is much smaller or larger than) most of the other values in a set of data.

ovum An unfertilised reproductive cell containing a haploid nucleus.

P

palindrome A sequence that reads the same backwards as forwards.

parallel evolution The independent evolution of similar traits in different species in response to similar selection pressures.

parapatric speciation The speciation that occurs in populations that are not geographicalally isolated, but where there is significant variation in habitat conditions within the range of the original population.

paraphyletic group A new group formed by descendants of a common ancestor.

parasite An organism that lives in or on another organism (a host) and benefits by obtaining nutrients by feeding on that organism.

parasitism An interaction between species where one organism, the parasite, feeds from another organism, the host. The host is harmed and the parasite benefits.

parental generation The first generation of individuals crossed when analysing genotypes. Also known as the P generation. The probable offspring from the parental generation are known as the first filial (F1) generation.

parental type A gamete that has the same alleles that are present in the parent. Also called a parental gamete.

patent right The right to make or sell something.

pathogen An agent that causes disease or illness to its host.

pedigree analysis The determination of the pattern of inheritance of a characteristic or condition by reference to a family tree in which the presence or absence of the characteristic is recorded over generations.

pelagic zone All of the water of the open ocean.

percentage cover The percentage of an area covered by a particular species. Usually refers to plants but may be used for colonial organisms like corals.

percentage frequency The percentage of quadrats in which a particular species occurs.

period One of several subdivisions of geologicalal time enabling cross-referencing of rocks and geological events from place to place. Eons and eras are larger subdivisions than periods, and periods can be divided into epochs.

personal protective equipment (PPE) The equipment specifically designed and used during activities to protect the user from hazards.

phenotype The character, trait or overall appearance of an organism as a result of the expression of its genes during development.

photoautotroph See photosynthetic autotroph.

photosynthesis The process by which plants and other photosynthetic organisms convert energy from sunlight into chemical energy for biological function. It occurs in plastids.

photosynthetic autotroph An organism that is able to synthesise organic compounds from inorganic using sunlight as an energy source. Also called a photoautotroph.

phylogenetic classification The classification of organisms based on their assumed evolutionary histories and relationships.

phylogenetic tree A summary diagram that shows how organisms are related and how they are assumed to have diverged during their evolutionary descent. Also called a phylogram.

phylogeny The assumed evolutionary relationships of species.

phylogram A summary diagram that shows how organisms are related and how they are assumed to have diverged during their evolutionary descent. Also called a phylogenetic tree.

phylum A group in the hierarchy of groups and sub-groups of living organisms. It is smaller than a kingdom and larger than a class.

pie chart A spherical display of either qualitative or quantitative data showing the proportion of each data set (measurement, independent variable, observation) as a part of the whole.

pioneer community The initial group of species that colonises new or disturbed terrain. It consists of a small number of pioneer species that are able to withstand harsh conditions.

plasmid Small, circular pieces of double-stranded DNA found in bacterial cells. Plasmids replicate independently of the bacteria's chromosomal DNA and are used in genetic engineering for creating recombinant DNA.

ploidy The number of full sets of chromosomes in an organism's karyotype. Haploid (one set, or n) and diploid (two sets, or $2n$) are the most common ploidy states, but other states are possible.

point mutation A mutation in which a single nucleotide base in the DNA or RNA molecule is changed.

pollinator An organism that carries pollen from one plant to another plant of the same species, facilitating the reproduction of the plant.

poly-A tail A long chain of adenine nucleotides added to mRNA during processing to increase the stability of the molecule.

polygenic A phenotype (characteristic) determined by the interaction of multiple genes.

polygenic inheritance When one characteristic is controlled by two or more genes.

polymerase A group of enzymes that catalyses the formation of polymers, in particular the formation of nucleic acid polymers by complementary base pairing with a template strand.

polymerase chain reaction (PCR) A laboratory technique used to amplify (make millions of copies of) a piece of DNA in a short period of time.

polymorphism Genetic variation within a population. The least common allele has to have a frequency in a population of 1% or more for it to be considered polymorphism rather than mutation.

polyphyletic group Organisms that have been grouped together in a clade but who do not share a common ancestor.

polyploidy The number of sets of chromosomes in a cell in which every chromosome has more than two copies; denoted xn, where x is the number of copies of chromosomes, such as $3n$ (triploid) or $6n$ (hexaploid) wheat varieties.

polytomy A node on a cladogram that has more than two branches of immediate descendants.

population A group of organisms of the same species that interact with each other.

population crash A very rapid decrease in the size of a population of organisms.

population explosion A very rapid increase in the size of a population of organisms.

positive selection The natural selection process that favours an allele or heritable trait.

precision Accuracy; exactness; exact observance of forms in conduct or actions. In science, exactness; how close two or more measurements of the same object or phenomena are to each other.

predation Killing living animals (prey) for food.

predator An animal that catches live prey for food.

predisposition Being likely or having the tendency to acquire a disease.

primary productivity The rate at which solar energy is utilised to fix carbon into organic molecules.

primary source The original source from which information was created or data was measured.

primary succession The series of changes in community composition that develops over time on newly formed terrain that has previously been uninhabited by living things, such as newly made volcanic rock.

primer A short strand of DNA or RNA that is able to bind or anneal to single-stranded DNA to create a region where DNA polymerase can join and initiate DNA synthesis.

processed data Data that has been manipulated to produce meaningful information; a set of displayed data in an appropriate form such as tables or graphs; required information that has been extracted from a set.

producer An organism that makes its own food, usually by photosynthesis.

productivity The contribution that plants make to the biomass of ecosystems.

profile diagram A diagram derived from data collected from the mid-line of a transect that shows a cross-sectional profile of the surveyed area. It displays elevation, zonation and the occurrence of organisms along the transect line.

programmed cell death The process by which a cell is genetically determined to die as part of its role in the organism.

prokaryote A unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle. Prokaryotes are divided into two domains: Archaea and Bacteria.

promoter region A specific nucleotide sequence in DNA that binds RNA polymerase and indicates where to start transcribing RNA.

pronucleus A cell nuclei before fusion forms the nucleus of a zygote.

prophase The first phase of mitosis where chromosomes condense and become visible under the microscope.

protein A biological polymer constructed from amino acid monomers. It performs structural, contractile, transport, catalytic and some hormonal functions.

protein synthesis A two-stage process involving transcription of the DNA and translation of the genetic code to form polypeptide chains.

purine A cyclical nitrogen-containing organic compound that includes adenine and guanine.

pyrimidine A cyclical nitrogen-containing organic compound that includes cytosine, thymine and uracil.

Q

quadrat A square, rectangular or circular frame of convenient size, used to mark out an area in which organisms are to be sampled.

qualitative data Information that is not numerical in nature.

quantitative data Numerical information (Taylor 1982).

R

***r*-selection** The process that occurs when population numbers are below the carrying capacity of an unstable environment and organisms produce large numbers of offspring that develop and fend for themselves from an early age, even though mortality rates are high and few offspring will achieve maturity.

***r*-strategist** An organism that practises *r*-selection.

random error An error that affects the measurement in an unpredictable way, resulting in an even variation (fluctuation) in measurements above and below the expected true value.

random selection The process of using a selection process that is not controlled by the experimenter.

range The variation in values between upper and lower limits on a particular scale or the largest and smallest measurements.

rapid speciation See speciation.

raw data Unprocessed and/or unanalysed data; data that has been collected without any additional processing (Taylor 1982).

realised niche The actual use of resources of an organism.

recessive phenotype A phenotype that is observed only in homozygous individuals; a trait or phenotype (encoded by an allele or gene) whose appearance is subordinate to a dominant trait.

reciprocal cross A cross in which a male of strain A is crossed with a female of strain B, and a female of strain A is crossed with a male of strain B.

recognition site The short sequence of DNA bases recognised and cut by a restriction enzyme; also called a restriction site.

recombinant DNA DNA that has been genetically engineered by joining fragments of DNA from two or more different organisms

recombinant gamete A gamete that carries a combination of alleles not observed in the parents, as a result of crossing over during meiosis.

recombinant plasmid A plasmid containing a foreign gene that has been inserted by the use of restriction enzymes and DNA ligase.

recombination In offspring, the formation of a new combination of alleles from the total alleles available from the parents.

redirect In science, to redirect an experiment is to modify the methodology to gain further insight into the phenomena observed in the original experiment.

reference list A list of resources that are cited or referred to in the document.

refine In science, to refine an experiment is to modify the methodology to obtain more accurate data or precise data.

regulatory gene A region of DNA that codes for transcription factors that control gene expression.

relative species abundance A measure of the relative proportions of each species within a community.

relative uncertainty The measured uncertainty displayed as a percentage of the measurement.

reliability In science, the likelihood that another experimenter will obtain the same results (or very similar results) if they perform exactly the same experiment under the same conditions (ACARA 2015c, Taylor 1982).

repeat trial The process of repeatedly conducting a process, activity or trial in an attempt to replicate the results.

replication The process of repeatedly conducting or choosing a process, activity or sample in an attempt to replicate the results, event or set up.

replication fork The point at which the complementary strands of the parent DNA unzip, or separate from one another, during DNA replication.

repressed A gene that is inhibited and cannot be transcribed.

research question A question that directs the scientific inquiry activity; it focuses the research investigation or student experiment, informing the direction of the research, and guiding all stages of inquiry, analysis, interpretation and evaluation.

restriction enzyme A type of enzyme, also called an endonuclease, that occurs naturally in bacteria and can cut DNA at a particular site (a recognition site); used in genetic engineering.

reverse transcriptase A type of polymerase enzyme used by retroviruses to copy their RNA genome into DNA; used in genetic engineering to copy messenger RNA (mRNA) into complementary DNA (cDNA).

ribonucleic acid (RNA) A nucleic acid made up of a sequence of ribose sugars and bases (adenine, cytosine, guanine and uracil) linked by phosphate bonds.

ribose A five-carbon sugar found in the RNA polymer chain.

ribosomal RNA (rRNA) The rRNA component of the ribosome.

risk assessment Evaluations performed to identify, assess and control hazards in a systematic way that is consistent, relevant and applicable to all school activities; requirements for risk assessments related to particular activities will be determined by jurisdictions, schools or teachers as appropriate (ACARA 2015c).

RNA ligase A ligase enzyme that joins together fragments of RNA.

RNA polymerase An enzyme that catalyses the synthesis of RNA, using an existing strand of RNA or DNA as a template.

RNA processing A modification of RNA before it leaves the nucleus, a process unique to eukaryotes.

root The root of a phylogenetic tree represents the beginning of an organism's ancestral lineage.

rooted tree The most basal ancestor in the lineage of a phylogenetic tee.

S

safety data sheet (SDS) A document that outlines specific details about items that are required to be considered when handling the item for experimentation.

saprophytic bacteria Bacteria that feed on dead or decaying organic matter. Saprophytic bacteria are decomposers.

scatterplot A graph that displays the measured values for two variables plotted along two axes.

scavenger An animal that feeds on dead animals.

scientific journal A book containing the entire collection of work related to an experiment or investigation.

scientific notation A measured value written as a mathematical expression, between 1 and 10 including decimals, displaying all significant figures, multiplied by 10 raised to a specific power.

scientific report A document that follows the report genre, systemically organising the main information and findings of scientific research or work.

Secchi disc A black and white disc used to measure turbidity.

secondary data Data collected by a person or group other than the person or group using the data (ACARA 2015c).

secondary source A source of information or data that was not created or measured by its author, but which cites another source.

secondary succession The series of changes in community composition that develops over time on terrain that has previously been inhabited by living things but which has suffered disturbance, such as fire or clearance by humans.

selection pressure An environmental factor that affects the survival and reproductive success of an individual based on their particular phenotype.

selective agent An environmental or introduced condition or factor that acts as a selection pressure.

semi-conservative DNA replication is described as semi-conservative because each daughter DNA molecule consists of one old and one newly synthesised strand.

sere A community that forms during the process of ecological succession.

sex chromosome A chromosome that is involved in sex determination. In humans, the sex chromosomes are the X and Y chromosomes. Also called an allosome.

sex determining region Y (SRY) A regulatory gene that codes for the SRY protein, which regulates the initiation of male sex determination in mammals.

sex-limited inheritance The inheritance of a trait that is expressed only in one sex, even though both sexes carry the gene. An example is haemophilia A, an X-linked trait that occurs almost exclusively in males.

sex-linked inheritance Inheritance related to genes that occur on sex chromosomes (X and Y in humans). An example is red–green colour blindness, which is caused by a mutation in a gene on the X chromosome.

sexual selection The difference in the ability of individuals to acquire mates. It typically involves contests between males or choice by females and leads to the selection of characteristics relating to mate attraction. Individuals that possess the desired characteristic are more likely to mate and pass on their alleles to the next generation. The trait, such as number of eye spot feathers in peacocks, is often an indication of overall health and fitness and other alleles of high adaptive value.

significant figures The digits recorded according to the precision of an instrument; the digits in a calculation that represent the precision of an instrument.

Simpson's diversity index A measure of species diversity, calculated using the formula:

$$\text{SDI} = 1 - \left(\frac{\Sigma n(n-1)}{N(N-1)}\right)$$

single nucleotide polymorphism (SNP) A single base change in DNA; when comparing gene sequences between individuals, the single base changes are called polymorphisms or SNPs.

sister taxa A group of organisms that arise from a terminal node of a phylogenetic tree.

somatic cell A body cell of an organism except for cells that give rise to gametes (eggs and sperm), cells that contain a full set of chromosomes.

somatic mutation A mutation that occurs in somatic, or non-gamete, cells of an organism. These types of mutations may affect an individual but cannot be passed on to their offspring. Cancer is a form of somatic mutation.

speciation The formation of a new species following a lineage splitting event. Speciation may result from geographical, anatomical, physiological or behavioural barriers to breeding, leading to divergence over evolutionary time, or may be rapid as a result of adaptive radiation.

species The basic group in the hierarchy of groups and sub-groups of living organisms. It is smaller than a genus. Organisms that are grouped into species usually closely resemble each other and can interbreed and produce fertile offspring.

species abundance The number of individual members of a species in a particular area.

species diversity All of the species in a community or ecosystem along with their relative abundance.

species evenness A measure of the relative proportions of each species within a community.

species richness The number of different species present in a community, ecosystem or region.

spermatocyte A cell in the testes that divides by meiosis to produce four sperm cells.

spermatogenesis The process by which spermatozoa are produced from precursor cells by meiosis.

spermatozoa Motile sperm cells that possess a flagellum.

spindle An arrangement of microtubules that binds to a centromere of a chromatid, enabling the chromosome to be divided equally between two daughter cells during mitosis and meiosis.

spliceosome A complex assembly that interacts with the ends of an RNA intron in splicing RNA, releases an intron and joins to adjacent exons.

splicing The removal of the introns during RNA processing.

spontaneous mutation A naturally occurring random change in genetic material.

stabilising selection A form of natural selection that favours intermediate traits; maintains or narrows the range of phenotypes.

standard deviation A calculated spread of data that estimates the quartile boundaries from the mean, assuming a normalised (bell) curve.

sticky-end restriction enzyme A type of restriction enzyme that makes a staggered cut in DNA to leave fragments with overhanging (or 'sticky') ends. The exposed bases of these sticky ends are then able to form complementary base pairs with nucleotides of other DNA molecules that have been cut with the same restriction enzyme.

stratified sampling A sampling technique where the population of an ecosystem is divided into smaller sub-groups, or strata. The division is conducted on the basis of the location of organisms within that ecosystem. The sub-groups are then sampled with a greater precision than would be possible without the grouping.

structural gene A gene that codes for proteins and RNA not involved in gene regulation. These genes could code for enzymes, protein channels, tRNA and many other types of proteins.

student *t*-test A calculation that produces a value that estimates the likelihood that two variables are not related.

subspecies Potentially interbreeding sub-groups of a species.

succession The process of gradual changes in an ecological community over a period of time. These changes are orderly and generally predictable in the absence of major disturbances.

symbiont An organism. involved in a symbiotic relationship.

symbiosis A close association between two different organisms, in which at least one of the organisms benefits from the association. Symbiosis includes mutualism, commensalism, and parasitism.

symbiotic relationship A relationship where two different organisms live and function together in a close association, to the benefit of at least one of them.

sympatric speciation The evolution of new species from a common ancestor, while occupying the same geographical range.

syncytium A cell containing two or more nuclei, resulting from a fusion of cells.

synonymous mutation A mutations that does not affect the amino acid sequence of a polypeptide chain. The different codon will still result in the same amino acid. These mutations are thought to affect the rate of RNA protein synthesis, shape and function.

systematic error An error in measurements caused by the design of a system (e.g. methodology) or instrument (without calibration) that results in the measurements shifting in a systemic direction.

T

Taq polymerase A type of heat-resistant DNA polymerase that is widely used in the polymerase chain reaction (PCR).

TATA box A common sequence of bases (TATAAA) in eukaryotic genes that codes for the promoter region.

taxon A taxonomic unit in the hierarchy of classification of organisms.

taxonomy The naming of groups recognised in a classification of organisms.

telophase The final stage of mitosis, where a nuclear membrane forms around the chromosomes at each pole.

template strand A strand of DNA or RNA used as a template for building a complementary strand of a precise nucleotide sequence.

temporal isolation The process where populations are separated by timing of activity or breeding cycles.

test cross A type of backcross in which an individual with a dominant phenotype is crossed with an individual of a recessive phenotype. A test cross is used to identify whether the individual with the dominant trait is homozygous or heterozygous.

thermophilic Relating to an organism that favours high temperatures (a thermophile).

thymine (T) One of the five nitrogen-containing bases found in DNA and RNA. The others are cytosine, guanine, uracil and adenine.

tolerance range The range between the upper and lower limits of any particular abiotic factor within which a particular species can survive.

trait A particular characteristic, e.g. hair colour and eye colour.

transcription The process by which a base sequence in DNA is used to produce a base sequence in RNA.

transcription factor A regulatory protein that binds to DNA and stimulates transcription of specific genes.

transect A line along which a biological survey is conducted.

transfection The process of artificially adding foreign DNA or RNA to a host cell.

transfer RNA (tRNA) An RNA molecule responsible for bringing specific amino acids to the ribosome for incorporation into a polypeptide during translation.

transformation efficiency The efficiency of cells taking up foreign DNA and expressing the genes encoded by the foreign DNA.

transformed A bacterium that has incorporated DNA from another organism into its own DNA, or taken up a plasmid containing foreign DNA.

transgene A gene or genetic material that has been transferred naturally or by genetic engineering techniques from one organism to another.

transgenic organism An organism with a genetic modification made by the transfer of specific gene(s) from another organism.

translation The process in which the base sequence of an mRNA molecule is used to produce the amino acid sequence of a polypeptide.

translocation mutation A type of block mutation that involves sections of two different chromosomes switching positions.

transposon A section of DNA that can move between chromosomes.

trend line A line drawn onto a graph to accurately display a relationship between the variables.

triplet A sequence of three nucleotides in DNA.

trophic cascade The series of ecological changes that occur in the food web when the top level predator is removed from an ecosystem.

trophic level The position of an organism in a food chain.

true value An ideal measurement of a phenomenon without error.

true-breeding The process of producing only progeny with a particular characteristic or trait seen in the parent. Also called pure-breeding.

tumour-suppressor gene A gene whose protein products inhibit cell division, thereby preventing uncontrolled cell growth (cancer).

turbidity A measure of the level of loss of clarity of water due to the presence of particles suspended in the water.

U

uncertainty Range of values for a measurement result, taking account of the likely values that could be attributed to the measurement result given the measurement equipment, procedure and environment (ACARA 2015c); indicators of uncertainty may include percentage and/or absolute measurement uncertainty, confidence intervals, inferential statistics, statistical measures of spread, e.g. range, standard deviation.

undirected network A diagram consisting of intersecting lines that show which species are interacting, but not the type of interaction.

unrooted tree A phylogenetic tree that does not require a common ancestor to illustrate the relatedness of its clades.

unweighted network A diagram consisting of intersecting lines that show which species are interacting but not the strength of those interactions.

uracil (U) One of the five nitrogen-containing bases found in DNA and RNA. The others are cytosine, guanine, thymine and adenine.

V

validity In science, the extent to which tests measure what was intended; the extent to which data, inferences and actions produced from tests and other processes are accurate (ACARA 2015c).

variety A botanical taxonomic group below that of subspecies, which has minor differences from other groups of that species or subspecies.

vector (1) An infectious disease: object or organism that transfers a pathogen from one host to another. (2) In molecular biology, a vehicle used to transfer foreign DNA into a cell (e.g. a plasmid, virus or liposome).

viability The ability of an organism to survive and reproduce.

viable offspring An organism that survives to maturity and is able to reproduce successfully.

virulence The severity or harmfulness of a disease or poison.

virus A microscopic non-cellular particle consisting of a DNA or RNA core with a protein coat, and perhaps a membrane envelope; able to penetrate cells, destroy them or interfere with their function, and multiply rapidly.

W

wild type The phenotype most commonly observed in a natural population.

X

X-linked A trait resulting from the inheritance of a gene on the X chromosome. An X-linked trait may be inherited from either parent, because both have an X chromosome.

xenotransplantation The transplant of an organ, tissue or cell from one species to another, such as from pig into human.

Y

Y-linked A trait resulting from the inheritance of a gene on the Y chromosome. A Y-linked trait is passed from father to son. It is never observed in females because they do not have a Y chromosome.

Z

zonation The division of plants and animals into specific regions based on environmental characteristics, e.g. light, nutrients, salinity.

zygote The diploid cell resulting from the fusion of an egg and sperm. It is the first stage of the development of a unique new organism.

Index

5' cap 271

abiotic environment 13, 47, 48, 71
  and biodiversity 91–119
absolute uncertainty e29, e30
  equation e30
abundance, measuring 50
abyssal pelagic zone 100
academic fraud e81
accuracy e27
achievability e62
acknowledging sources e91
adaptation 452, 453, 457
adaptive radiation 486
adenine (A) 250
adenosine triphosphate (ATP) 133, 134, 147
agriculture 384, 386, 387–92, 470–2
air 131
algae 135, 136, 187
algal blooms 105, 115–16, 186–7
allele frequency 340, 452, 453
  and changing environmental pressure 456–7
  equation 454
  in the field, calculating 455
  in a gene pool 454–9
alleles 264, 316, 334, 337, 343, 344, 348
  new, by mutation 457–8
allelopathy 163
allopatric speciation 479–81
allosomes 317, 327
amensalism 163
amino acid sequences, differences in 28–9
ammonification 153
ANAE (Australian National Aquatic Ecosystem) 24
  classification system 21, 24
analysis of evidence criterion e55
anaphase 309, 314, 315
aneuploidy 327, 329, 330–1
anneal 406
anomalies e39, e80, e83–e84
answerability e62
antibiotic resistance 383, 467
anticodon 269
antiparallel 251
ants, red fire 165
ants, yellow crazy 87
APA (American Psychological Association) style e91
apoptosis 419
aquatic biomes 130
aquatic ecosystems
  ANAE classification system 24
aquatic environments 100–6, 130
  depth 102, 103
  ecosystems variability 109
  light penetration 102–3
  turbidity 102–3, 104
aquifers 100–1, 150
Archaea 12
arocentric chromosomes 255, 257
arthropods, identifying 53
artificial selection 454
artificial transformation 401–2, 403
assimilation 147
atmosphere 131
auger 96
Australia *see also* Daintree *entries;* Kati Thanda–Lake Eyre; Queensland
  as biodiverse 74
  bioregion framework 18–21
  classifying plant communities in 22, 23
autism 384
autosomal co-dominant inheritance 347–8
autosomal dominant inheritance 344–7, 358–9
autosomal recessive inheritance 358
autosomes 258, 317, 327, 343
autotrophs 133, 134–5

background extinction 441, 443
  rates 443, 444
Bacteria 12
bacteria 5, 153, 398–9, 467–8
bacterial transformation 401, 404
bacteriophages 394
baldness in men 352–3
banksia, coast 480–1
bar graphs (or charts) e37–e38, e90
barriers, learning e8–e9
basal cover 75
base 249
Basic Local Alignment Search Tool (BLAST) 381
bedrock 93
belt transect 48
benthic region 100
bias e80, e81
  minimising 50
  sample e83
  selection e67
biased sampling 50
binary fission 301
binomial 6
  system 6–10
biodiversity 69–126, 70
  and abiotic environment 91–119
  and area size 99
  describing 3–4
  explained 6
  and interconnectedness of life 1–243
  and isolated environments 101
  loss of 390
  marine 74
  measuring 74–83
  and species interactions 84–8
  types 70–4
biofilm 401
biofortification 388
biogeochemical cycling 149–53
biogeographical regions 18
bioinformatics 380–5
  applications 382–4
  biological implications 382, 389–90, 392
  ethical implications 382, 391–2
  social implications 382, 390–1, 392
biological classification 6, 10–12
biological diversity 6
  recording 6
biological fitness 454
biological implications 382, 389–90, 392
biological species 7
biology
  assessment toolkit e1–e124
  learning e7–e15
  mathematical basics for e22–e24
  skills e1–e124
  types of investigations e56
biomass 137
  measuring 137–8
  pyramids 146, 147, 148
biomes 71, 72–3, 74, 91, 129, 130, 131
bioregions 18
biosphere 6, 129, 131
biotechnology 379–430
  success rates and limitations 410–11
biotic environment 13
bioweapon 383
black soils 98
block mutations 327–9
blood types 339
blowfly 470–2
blue whales and krill relationship 147
blunt-end fragments
  ligation 398
blunt-end restriction enzymes 395
books e102
bottleneck effect 459, 491
brackish environments 74, 130
branch 35
branching key 53
branching points *see* nodes
Brisson, Mathurin Jacques 9
Bt (transgenic) cotton 388, 410–11
business practices 391

calibrated salinity meter 98
calibration of equipment 50
cane toads 212–13
canopy cover 23, 75, 76
carbon cycle 150–1
carbon dioxide 192
  and alternative energy source 383
  levels 201–2, 209–11
  utilisation 139
carbon fixation 134, 135, 136
carcinogens 291
carnivores 140, 491
carriers 350
carrying capacity 185
Cas proteins 384
case studies
  abiotic influences on green sea turtles 107–8
  algal succession on a coral reef 115–16
  applied phylogeny and SARS 41
  are humans causing the sixth mass extinction? 443–4
  cassowary as a keystone species 168–9
  Drosophila on verge of speciation 483–4
  fossil teeth and environment 203
  the Great Barrier Reef 109–12
  keystone species of tropical Queensland 170–1
  phylogenomics for Darwin's finches 492
  positive and negative selection and context 461–2

positive selection in high-altitude Tibet 465–6
radiation of flightless birds 449
ray-finned fish evolutionary uncertainty 42
selection in rock pocket mouse 473
species interactions in Shark Bay 85
species interactions on Christmas Island 84, 85, 86–8
whale and hippo 448
cataloguing notes e107
cell cycle 301–3
cytokinesis 302, 310
eukaryotic 302–3
interphase 302, 303, 315
mitosis 302, 308–9
cell plate 310
cell replication 300
in eukaryotes 301–2
in prokaryotes 300–1
and variation 299–324
cellular respiration 133–4, 192
equation 147
central tendency, measures of e43
centrioles 309
centromere 266, 309
character table 34
Chargaff, Erwin 259
Chargaff's rule 259
chemical codes e70
chemoautotrophs *see* chemosynthetic autotrophs
chemosynthesis 135
chemosynthetic autotrophs 134, 135
chiasma 316
chlorophyll 136
chloroplasts 136, 137
Christmas Island
species interactions on 84, 85, 86–8
chromatids 255, 309
chromatin 282
chromosomes 249, 254–8
abnormality 326–33
number, and genetic variation 457
pair of X and Y 257
pair of Xs 257
things to look at 326
CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) 117–18
clade 16, 36, 37
cladistics 16
cladograms 38
claim e95
classifying elements of the e95–e96
defined e95
and ISMG e95
understanding the e95–e96
class 11
in Linnaean system hierarchy 11
classification 3–67, 6
biological 6
defined 5
ecosystems 17
importance 5–6
in practice 46–55
processes 27–45
climate 91–3, 129, 130, 453
climax community 192–3
co-dominance 337
coast banksia 480–1
coastal environments 74, 100
coding DNA 268, 385
coding strand 270
codon 265, 266–7
coefficient of determination e82
coenocyte 310
coevolution 490
column graphs *see* bar graphs
commensalism 162
common ancestor 445
common versus scientific language e87
community 129, 131
comparative genomics 445
compensation point 192
competence 401
competition 13, 164–5, 177–8, 453
competitive exclusion 159
complementary base pairing 251
complementary DNA (cDNA) 405
concept maps e13
concise language e89, e90
condensation polymerisation 250
confidence interval e45–e47, e52
calculating, worked example e46–e47
conservation of ecosystems 51
constitutive genes 278
consumer organisms 74, 133
consumers 74, 133
continuous variation 339
control group e67
controlled variables e57, e59, e61, e65
controls e67
convergent evolution 487–8
coral reefs 115–16, 130
correlation, common and scientific meaning e87
counting criteria 50
cover, measuring 50
Crick, Francis 259, 260
CRISPR 384–5, 410
cross 343, 366–8
cross pollination 389
crossing over 314, 316, 370
crowding 179
cystic fibrosis 289
gene 257
cytokinesis 302, 310
cytosine (C) 250

Daintree National Park 21
Daintree rainforest 92–3, 109, 129
Darwin, Charles 433–4, 456, 485, 486
Darwinian theory 434
data
analysing e81–e84, 51
biological, difficulties e42
capture rate e69
collection e76, e77–e79, 49
continuous e65
discrete e65
distorted presentation e40
electronic e68–e69
ice-core 212
missing e39
nominal e65
ordinal e65
personal, issues storing 382
presentation 49
processed e28, e36, e81
processing and analysing e85
qualitative e65, e66
quantitative e65, e66
raw e28, e35, e43, e61, e65, e80, e81
recording, in your journal e110–e111
recording numerical e27–e28
relevant e77, e79
researching published e56
secondary e100
sufficient e77–e78
test e16
deciduous plants 76
decomposers 140, 141
degenerate 267
deletion mutations 328
denitrification 153
denitrifying bacteria 153
density, measuring 50
density-dependent factors 177–80
density-independent factors 180–2
deoxyribonucleic acid (DNA) *see* DNA (deoxyribonucleic acid)
deoxyribose 249
dependent variable e36, e59, e61, e98
detritivore and decomposer food chain 143–4
detritivores 140, 141
detritus 140, 144
deviations e114
dichotomous keys 52
dideoxynucleotide 415
dihybrid cross 366–8, 369
dinosaurs 438
diploid 256, 263
directional selection 463, 464
discrete variation 339
disease 13
disruptive selection 463, 464
divergence points 36
divergent evolution 485–7
DNA (deoxyribonucleic acid) 9, 11, 12
see also coding DNA; complementary DNA (cDNA); gene, expression; gene, regulation; genes; guide DNA (gDNA); mitochondrial DNA (mtDNA); noncoding DNA; recombinant DNA
amplification 405–7
and chromosomes 254
circle sequencing 422–3
discovery 259–60
eukaryotic versus prokaryotic 252
explained 249
foreign 401–3
helicase 304
hybridisation 30–1
ladder 409
ligase 397–8
manipulating 385
nuclear, inheritance 29
polymerase 304
profiling 417–20
repair and mutations 292
replication 247–98, 303–4
replication, direction 305
and RNA, differences 254
satellite 417
sequences 32, 380, 421, 452

sequencing 30, 31, 381, 382, 414–17
structure 249, 251–2
thermocycler 406
working with fragments of 394–404, 407–9
domains 11
in Linnaean system hierarchy 11
three 12
dominance 337
dominant phenotype 337
double-helix 251
Down syndrome 330
drug resistance 468–9
Duchenne muscular dystrophy (DMD) 422
duplication mutations 328

ecological change
and human activity 205–15
using fossil record 200–5
ecological niche 157
ecological pyramid of energy 146
ecological surveying techniques 48
ecoregions 17
ecosystems 17
biogeochemical cycling 149–53
and the biosphere 131
change in 191–217
classifying 17–24, 46–51
coastal marine 73
conservation 51
contain habitats 132
diversity 71–4
dynamics 127–243
explained 129, 131
four classification types 21–4
functioning 157–75
Queensland regional 19–21, 22
species interactions within 84, 85, 86–8
succession 109, 191–200
variability 109
ectoparasites 162
ectothermic organisms 105
editing e90, e115
electrical conductivity 97, 98
electronic data
acquisition e68–e69
logging e68–e69
electroporation 401
emergent layer 22
emigration 183
endoparasites 162
endosymbiosis 436
energy transfer 133–56
in organisms 133–40
through ecosystems 140–8
environment
carrying capacity 186–7
coastal 74, 100
and fossil teeth 203
freshwater 100, 104–6, 130
impacts of GMOs on 391
isolated 101
marine 74, 100
organising 129–32
terrestrial 91–9, 130
environmental selection pressures 453–4
environmental toxins, resistance 467–8

eon 436
epigenetic inheritance 282–3
epigenetics 282
epochs 436
equilibrium 185
equipment e68, e75–e77, 50
calibrated salinity meter 98
pH probe 98
Secchi disc 103
sonar 103
turbidity tube 103
eras 436
error e27, e29
identifying e80–e81
instrumental e30
in methodology e83
minimisation 50
minimisation, and instruments e75
random e27, e29, e83, 50
random sampling e49
rate e50
reducible random e81
in results, identifying e80–e81
results during meiosis 331
sampling e49
and significant *P* value e49–e50, e52
standard e44–e45
systematic e27, e29, e81, e83
estuaries 74, 130
ethical considerations e71–e72, 382, 391–2
ethics e71
Eukarya 12
eukaryotes 12, 273, 277, 301–2
eukaryotic cell 252, 269, 273, 277, 302–3, 398, 402–3, 436
eukaryotic genes 267
eukaryotic organisms 256
EUNIS (European Nature Information System) 24
habitat classification system 21
eutrophication 196, 197
evergreen plants 76
evidence
criterion, analysis of e55
interpretation of e64
evolution
defined 434, 475
explained 433–4
intervention in 392
theory of 15, 433–4
evolutionary
change 434
history, major episodes 436–8
radiation 438–40, 456
relatedness 445–9
splits 36
timescales 439
trees *see* phylogenetic trees
examination e6, e116–e124
after e116, e124
preparation e117–e121
reading time e122
sitting e121–e124
summary e6, e116
writing time e122–e124
exons 267, 417
experimental group e67
exponential growth 184–5

extend e57
extension e57, e58
extinction 440–4, 491
extraneous variables e82, e83
eyes 488

F1 generation 343, 345, 346, 348, 366–70, 372
F2 generation 343, 345, 367–9
family 5
in Linnaean system hierarchy 11
fecundity 440, 453, 454, 457
fertilisation 312
fieldwork, conducting e56
finches, Galapagos Island 456–7, 485–6, 492–4
first person narrative e90
fixed 460
flash cards e117–e118
flystrike 470–2
food chains 141–4, 148
food webs 141, 144–5
forest 80
form 8
fossil *see also* microfossils
defined 436
as indicator of atmospheric gas 201–2
record 200–5, 438, 440
founder effect 459
Fox, George 12
frameshift mutation 289
Franklin, Rosalind 259, 260
freshwater environments 100–1, 104–6, 130
fundamental niche 158–60

Galapagos Island finches 456–7, 485–6, 492–4
gametes 256, 312–14, 316–17, 318, 343
recombinant 371–2
gel electrophoresis 407–9, 415
gene
cloning 397
editing 384
expression 264, 268–73
flow 458–9, 470
mapping 372
names, writing 335
pool 454
probes 30
regulation 266, 277–85
therapy 383
genes 249, 264, 337, 343
alternate splicing 281–2
eukaryotic 267
and genetic code 265–7
and genomes and alleles 263–4
linked 369–72
master regulatory 279–81
sex determining 280–1
tumour-suppressor 281
genetic
code 265–7
differences between organisms 30–1
diversity 70, 491–4
drift 459
expression 263–76
isolation 476–7
variation via polyploidy 457

genetically modified organisms (GMOs) 384, 385–92
genetically transforming cells 401–4
genome 263–4, 386
  mapping 418–20
genotypes 334–5
genus 5, 17
  in Linnaean system hierarchy 11
geographical isolation 476, 477, 479–80, 482
geological timescale 436–8
germ cells 312
germline 385
  mutations 286
glucose 135, 136
golden rice 388
Gondwana 486
graphs e36–e41, e90
  distorting the truth e40
  features e38–e39
  missing data e39
  presenting data in e36
  types e36–e38
Great Artesian Basin 100–1, 150
Great Barrier Reef 73, 109–12
great white shark 166–7
green fluorescent protein (GFP) 399
green sea turtles 107–8
greenish soils 98
gross primary productivity 137
ground cover 75
guanine (G) 250
guide RNA (gRNA) 384, 385

habitat 132, 157
  fragmentation 207–8, 477, 491
haemoglobin 264
haemophilia 350
haploid 256, 263, 314, 318
HAZCHEM e70
headings e88–e89
heat 291–2
hemizygous 334
herbivores 140
hereditary 'factors' 343
heredity and continuity of life 245–521
heterogametic 258
heterotrophic organisms 92
heterotrophs 133, 139–40
heterozygote 334
hierarchy 10
high-impact learning
  characteristics e7–e8
  strategies e7–e8
histograms 340
histones 282
HIV (human immunodeficiency virus) 460
Holdridge life zone classification scheme 21–2
homeotic genes 279
homogametic 258
homologous chromosomes 257–8, 314, 326, 445
homozygosity 208
homozygote 334
horizon 93
Hox genes 279–80
human
  activity 205–15, 452
  baldness in men 352–3
  blood types 339
  consumer rights and choice 391
  equal access to food 391
  height 331, 339
  karyotype 327
  lineage 438
  microbiome 384
  positive selection pressure of HIV in 460
  and sixth mass extinction 443–4
Human Genome Project 381–2, 419–20
human-made changes 182
humus 94
hunger, ending 390
hybrid inviability 476, 478
hybrid sterility 476, 478
hybrids 345
hydrosphere 131
hypothesis
  defined e61
  examples e63
  formulating a e61
  null e47, e49, e58, e61
hypoxic 104

identification keys e71
imagination e9
immigration 183
in-text citations e91
incomplete dominance 338
increasing abstraction representations e12
independent variable e35, e59, e61, e67, e98
individual 129, 131
induced 278
induced mutations 290–1
inducible operon 278
infectious diseases 13, 180
inference e85
inferential statistic e47
influenza, new strains 470
ingroups 35
inheritance 325–77
  patterns, recognising 358–63
inorganic compounds 134
insecticide resistance 470–2
insects, microevolution in 470–2
insertion mutation 328
instrument uncertainty e82
instrumental error e30
  worked examples e31
instrumental precision e82
instruments e75–e77
  lower and higher precision e76
  and significant figures e32, e33
internet information e103
interphase 302, 303, 315
interspecific competition 164, 177
interspecific hybrids 8–9
intertidal zone 74, 100
intraspecific competition 164, 178
introns 267
inversion mutation 328
ISMG (instrument-specific marking guide) e54, e58, e91–e92, e95, e97, e98, e113, e114
isolated environments 101

journal *see* scientific journal (your)

K-strategists 12, 13
karyotype 326–7
Kati Thanda–Lake Eyre 92–3, 109
Kell gene 257
keystone species 166–71
kingdoms 6
  five 11
  in Linnaean system hierarchy 11
Klinefelter syndrome 331
Köppen climate classifications 24
krill and blue whales relationship 147

lac operon 278–9
lac repressor 279
lacI 279
lactose 278
lacZ gene 404
lagging strand 305
lake *see also* freshwater
  succession and eutrophication 196–7
Lake Eyre *see* Kati Thanda–Lake Eyre
Lake Yumberarra 112–14
land clearing 206–9
Law of Independent Assortment 366, 369
law of large numbers 50
Law of Segregation 343, 366
leaching 92, 94, 98
leading strand 305
leaf 35
leaf cover 75
learning
  barriers e8–e9
  biology e7–e15
  consolidating e117–e121
life on Earth, continuity 431–521
ligases 397–8
ligation 397
light energy 146
light utilisation 136, 137
limiting factor 177
Lincoln index 182
line graphs e36–e37, e90
line transect 48
lineages 12, 35, 40, 438, 476
linkage 369
linked genes 369–72
Linnaean system 10–11, 15, 16
Linnaeus, Carl 6, 7, 9, 11
literacy, scientific e87–e88
literature review e113–e114
lithosphere 131
littoral zone 74
loams 95
locus 264
log book 49
logistic growth 185–7
Lord Howe Island 481–2
Lord Howe Island stick insect 482
lyse 401

macroevolution
  explained 435, 475
  processes 485–90
macromolecules 380
macroscopic representations e8, e10, e13, e14

malaria 461–2
mandatory practical
comparing plant diversity 218–20
comparing species diversity of communities 120–2
modelling evolution and measuring population genetics 498–501
reporting on an ecosystem 56–60
mangroves 74, 193–5
marine environments 100
abyssal pelagic zone 100
benthic region 100
coastal 74, 100
intertidal zone 74, 100
pelagic zone 100
mass extinctions 441, 443–4
massively parallel sequencing 416
mate availability 453
materials e68
mathematical basics e22–e24
matrix keys 53
maximum parsimony 34, 35
Mayr, Ernst 7, 8
mean e28, e43
equation e43, e52
standard error of e44–e45
measured variables e57, e59, e65
measurement *see also* scientific notation (SI)
and significant figures e32, e33
uncertainties e27–e34
measures of central tendency e43, e82
median e43, e52
medicine and bioinformatics 382–3, 384–5
meiosis 313–15
and mitosis, differences 314
results of errors during 331
stages 314–15
meiosis I 313, 314, 315
meiosis II 313, 314, 315
memory
aids e117–e118
triggers e118
Mendel, Gregor 343
Mendel's first law 343
mesopredators 166
messenger RNA (mRNA) 253, 265, 267, 268–73, 287
metabolic processes 147
metacentric chromosomes 255, 257
metaphase 309, 314, 315
methodology e64, e72
microbes, microevolution in 467–9
microbial genome analysis 383
microevolution 434, 452, 456, 458
examples 464–73
microfossils *see also* fossil
as indicator of habitat change 205
as indicator of temperature change 204–5
microhabitat 17, 132, 481
microinjection 403
microsatellite 405
microscopic representations e8, e10, e11, e13, e14
migration 183
mitochondrial DNA (mtDNA) 17, 29
mitosis
cell cycle 302, 308–9
and meiosis, differences 314
mode e43, e52
model, designing a e56
modern extinction rates 443–4
modification e57, e58
modify e57
molecular homology 445
molecular medicine 382, 384–5
molecular methods 421
Monkey Mia beach 85
monogenetic traits 338–9
monogenic 334
monohybrid cross 343
monomers 249
monophyletic groups 37
morphological features 8
morphology 7, 32
mortality 183
mosaic 410
mule 9, 478
multi-access keys 53
multiple crosses 366–8
multiplex ligation-dependent probe amplification (MLPA) 421–2
mutagens 286, 291–2
mutation rate 28
mutations 28, 32, 34
beneficial 290
block 327–9
causes 290–2
deletion 328
detecting 421–3
and DNA repair 292
duplication 328
explained 286
frameshift 289
in genes 286–93
germline 286
induced 290–1
insertion 328
and microevolution 457–8
non-synonymous substitution 288
nonsense 289
point 287
and restriction enzymes 396
somatic 286
spontaneous 290
synonymous 287
translocation 329
types 287–9
mutualism 161

name changes 9–10
naming species 7–8
nanopore sequencing 416
natality 183
natural disasters 182, 477
natural selection 433, 453–4, 463–4
natural transformation 401, 402–3
natural variation e67, e82, e83
naturalist 433
negative selection 460, 461
net primary productivity 138
networks 84
niche 157–60
niche partitioning 160
nitrification 153
nitrifying bacteria 153
nitrogen cycle 151–3, 192
nitrogen-fixing bacteria 153
nitrogenous bases 250
node 35, 36, 39, 40
non-disjunction 329–30
non-infectious diseases 13
non-synonymous substitution mutations 288
noncoding DNA 266, 454, 487
nonsense mutations 289
normal distribution e42
curve e43, e44
Normalized Vegetation Index 77
notes
cataloguing e107
notetaking e107–e111
summarising (for exam) e118–e119, e120–e121
nucleic acids 249–50
nucleosomes 282
nucleotide sequences, DNA 30, 31, 32
nucleotides 249–50
null hypothesis e47, e49, e58, e61
Nullarbor Plain 101

observation e66
Occam's razor 34
ocean 74, 106, 130, 131
Odum, HT 148
Okazaki fragments 305
omnivores 140
oncogenes 291
online searches e103
oocytes 318
oogenesis 318–19
and spermatogenesis, comparison 319
open systems e61, e67, e75–e77, e77–e78
conducting an experiment in e75–e77, e77–e78
operator 278
operon 278
order 11
in Linnaean system hierarchy 11
orders of magnitude e17–e21
and prefixes e18–e19
organ transplantation 387
organic compounds 134
organising notes e107–e111
organisms
classifying 6–17
consumers 74, 133
ectothermic 105
eukaryotic 256
genetically modified 385–92
heterotrophic 92
interactions 129–32, 140–1
morphology 7
photosynthetic 100
producers 74, 133
sex determination 258
that may pose a risk e71
origin 300
other feeders *see* heterotrophs
outgroup 35
outliers e39, e80, e83, e84
ovum 318
oxidation 135

P value e49, e52
interpreting e50–e51
significant, and error e49–e50, e52

palindromic 384
parallel evolution 489
parapatric speciation 482–4
paraphyletic groups 37
parasite–host food chains 143
parasites 13, 140, 468–9
parasitism 162–3, 179
parental generation 343, 344
parental type 370
Patau syndrome 331
patent rights 385
patents 391
pathogens 13
Pearson correlation coefficient e82
pedigree analysis 356–7, 363
pedigree charts 356–7
  conventions and symbols 356–7
peer-reviewed scientific journals e101
pelagic zone 100
percentage cover 75–9
  calculating 78–9
percentage frequency 79–80
  calculating 79–80
periods 436
personal protective equipment (PPE) e70
personalised medical treatment 383
persuasive writing e90
pH
  freshwater 106
  marine water 106
  soils 93, 95
phasmid 482
phenotypes 335–9, 343
  co-dominance 337
  complete dominance 337
  dominance property 337–8, 344
  explained 335
  fittest 479
  incomplete dominance 338
  influences on 335–7
  most advantageous 453–4
  and selection pressure 452
phenotypic
  ratios 344
  selection 460–4
  variations 452
photoautotrophs *see* photosynthetic autotrophs
photograph e75
photosynthesis 133–4, 136, 192
  equation 136
photosynthetic
  autotrophs 134, 135
  organisms 100
  pathways 136–7
  protists 135
phylogenetic classification 15–17
phylogenetic trees 16, 17
  building 33–5, 445–7
  different forms 38–40, 487
  explained 32, 33
  parts 35–7
  preferred tree 34
  three ways to group taxa in 36
phylogenies *see* phylogenetic trees
phylogeny 15, 16
  applied, and SARS 41
phylograms 38, 40
phylum 11
  in Linnaean system hierarchy 11
physical publications e102
pie charts e38, e90
pioneer community 192–3
plagiarism e91, e108
plant communities, classifying Australian 22, 23
plants, green 135, 136
plasmids 252, 398–9
ploidy 256
point mutation 287
pollinators 165
poly-A tail 271
polygenic 338
  inheritance 339
  traits 338, 339
polymerase chain reaction (PCR) 405–7, 421, 422
polymerases 405
polymorphism 396
polynucleotide chains 250
polyphyletic groups 37
polyploidy 457
polytomies 40, 447
population 129, 131
  crash 187
  dynamics 176–90
  explosion 184
  growth 183–7
  growth, equation 184
  size, calculating 182–3
  size, factors affecting 176–82
  size, formula 183
  variation within 452–4
pores 93, 94–5
positive selection 460, 461
practice questions e119
practicising e119–e121
precision e27, e28
  analysing e82
predation 13, 14–15, 164, 178–9
predator 140
  abundance 453
predator–prey
  food chains 142
  relationships 490
predisposition 381
prefixes, SI *see* scientific notation (SI)
presenting scientific ideas e90
preventative medicine 383
primary producers 134, 141
primary productivity 138
primary research
  examples e56
primary sources e100
primary succession 197–9, 200
primers 405
producer organisms 74, 133, 134
producers 74, 133, 134, 147
profile diagram 48
programmed cell death 419
prokaryote kingdom 12
prokaryotes 135, 273, 277
prokaryotic cell 252, 273, 300–1, 398, 401–2, 436
promoter regions 267
pronucleus 403
prophase 309, 314, 315
protein synthesis 249, 268
  in eukaryotes 269, 273
  in prokaryotes 273
proteins 249
Punnett squares 344–7, 369
purines 250
pyramids of biomass and energy 146, 147, 148
pyrimidines 250

quadrat maps 77
quadrats 49–50, 76–8, 80
Queensland 19–21 *see also* Australia; Lake Yumberarra; Lord Howe Island; Raine Island
  Daintree National Park 21
  Daintree rainforest 92–3, 109, 129
  and Great Artesian Basin 100–1
  Great Barrier Reef 73, 109–12
  keystone species of tropical 170–1
  land zones 20–1
  regional ecosystems 19–21, 22
  soil variation 98

r-strategists 12–13
Raine Island 107
rainforests, tropical 130
  see also Daintree rainforest
random errors e27, e29, e81, e83, 50
random-number generators 50
random sampling error e49
random selection e67
randomisation e67
randomised sampling techniques 50
range e27, e42–e43, e52
rationale e63–e64
ray-finned fish, evolutionary uncertainty 42, 447
realised niche 158–60
recent evolution 445
recent extinction 442
recessive phenotype 337
reciprocal cross 349
recognition site 394
recombinant DNA 385, 398–400
recombinant gametes 371–2
recombinant plasmid 399
recombination 316
recording notes e107–e111
red fire ants 165
red soils 98
redirect e57
redirection e57, e58
reducible random errors e81
reference list e91, e99
refine e57
refinement e57, e58
regulatory genes 278
relatedness between species 27–31
relative species abundance
  see species, evenness
relative uncertainty e29, e31
  equation e31
relevance e62
relevant e98, e99
reliability 48, e67, e104–e105
reliable interpretation e85
repeat trials e67
replication e67

replication fork 304
report (for research investigation) e112–e116
editing e115
literature review as e113–e114
presenting e112
structure e114–e115
representations
in biology e8, e9–e15
and effective learning e13–e15
increasing abstraction e12
repressed 278
reproduction 453
methods, classifying by 12–13
research
and ethical considerations e71–e72
and planning e57–e74
and planning, criterion e94
primary e56
question *see* research question
relevant scientific information e63–e64
and risk assessment e69–e71
secondary e56
research investigation e5, e93–e115
ISMG e94, e97, e98
research and planning criterion e94
summary e5, e93
writing a report for e112–e116
research question
developing e59–e61
developing the rationale e63–e64
evaluating your e62
examples e60, e63
explained e59
forming a e96–e97
refining the e97–e98
structure e60
resources
choosing suitable e99–e106
overview e103
teachers as e117
restriction enzymes 394–8
results e80–e86
analysing e81–e84
common and scientific meaning e87
identifying errors e80–e81
interpreting e85
retroposons 487
reverse transcriptase 405
revising e119–e121
revision summary e120–e121
ribonucleic acid (RNA) *see* RNA (ribonucleic acid)
ribose 249
ribosomal RNA (rRNA) 253, 269
ribosome 269, 271
risk assessment e69–e71, e77
rivers *see* freshwater
RNA (ribonucleic acid) *see also* gene, expression; gene, regulation; genes; guide RNA (gRNA); messenger RNA (mRNA); ribosomal RNA (rRNA); transfer RNA (tRNA)
and DNA, differences 254
explained 249
ligase 397
polymerase 267
processing 271
sequences 32
structure 253–4
viruses with 470
rocks 131
root 35
rooted phylogenetic trees 38, 39–40

safety data sheets (SDS) e70–e71
salinity meter 98
salt-tolerant wheat 388
saltmarsh *see* mangroves
sample
bias e83
common and scientific meaning e87
rate e69
size e42, e67–e68
sampling error e49
Sankey diagram 145–6
saprophytic bacteria 153
satellite DNA 417
satellite images 77
scatterplots e36
scavenger 140
science as a human endeavour
CITES 117–18
discovery of DNA 259–60
Indigenous peoples' knowledge of ecosystems 214–15
issues related to GMOs 389–92
LANDSAT satellites 54
scientific ideas, presenting e90
scientific inquiry process e54
scientific journal (your) e64, e72, e80, e85, e87, e95, e96
notetaking e107–e111
notetaking, worked example e108
personal explanations e109
personal interpretations e109
results, data and evidence e110–e111
scientific journals (published) e101–e102
scientific literacy e9, e14, e87–e88
scientific notation (SI) e17–e18, e21
chart of 12 official prefixes e19
converting to, skillbuilder e17–e18
converting to, worked examples e18, e20
unit symbols e25–e26
units e25–e26
scientific report (for student experiment) e55, e64, e72, e85
communicating concepts e88
editing e90
parts e88
sections e89
writing e87–e92
scientific writing e87–e88, e89–e90
sea floor 100
sea water 74, 104
seas 106
Secchi disc 103
secondary carnivore 140
secondary consumer 140, 141
secondary research, example e56
secondary sources e100
secondary succession 199, 200
selection bias e67
selection pressures 452
selective agents 452
selective breeding 452
self-feeders *see* autotrophs
semi-conservative 304
sere 192
sex chromosomes 258, 280–1, 317, 327
sex-determining region Y (SRY) 281
sex-limited inheritance 353
sex-linked inheritance 348–53, 359–61
ruling out 361–3
sexual reproduction 312
and gametes 312–14
and genetic variation 316–17
sexual selection 478–9
Shannon-Wiener index 82–3
shark, great white 166–7
short tandem repeats (STRs) 417
SI units, prefixes and symbols *see* scientific notation (SI)
sickle-cell anaemia 288–9, 461–2
significance, common and scientific meaning e87
significant figures e32, e33
significant *P* values e49–e50, e52
Simpson, Edward H 83
Simpson's diversity index e83, 83
calculating 83
single nucleotide polymorphisms (SNP) 396–7, 445
sister taxa 35
skillbuilder
converting between units e19–e20
from decimal to scientific notation e17–e18
determining significant figures e32
evaluating sources e104–e105
skin cancer 291
slopes, steep 94
social implications 382, 390–1, 392
social inequality 390
soil core 96
soils
classification 97
and climate 92
colour 98
leaching 92, 94, 98
measuring characteristics 96–8
nutrients 93, 95–6, 98
as part of lithosphere 131
pH 93, 95, 98
porosity 93, 94–5
quality, major determinants 93–6
salinity 93, 96, 97–8, 181, 208–9
salinity, tests 97–8
structure 93, 94, 97
thickness 93–4
waterlogged 98
solar energy 135, 137
somatic cells 256, 312
somatic mutations 286
sonar 103
sources
acknowledging e91
evaluating e104
primary e100
recording e91, e111
secondary e100
Spearman's rank correlation e82
Specht, R. L. 22
Specht's classification system 21, 22–3, 49
speciation
defined 438, 475, 476, 479

isolating mechanisms 476–9
modes 479–84
modes, compared 482
processes 475–6
species 5, 17
abundance 81
biological 7
conservation status 442
defined 476
determining relatedness 27–31
diversity 71
evenness 81–2
evenness, calculating 81–3
evenness, equations 82–3
evenness, formula 82
explained 7–8, 475
extinction 440–4
genome mapping 418–19
identification 49, 51–3
interaction methods, classifying by 13–15
interactions 84, 85, 86–8, 160–5 *see also* organisms, interactions
in Linnaean system hierarchy 11
naming 7–8
new, introduction 206, 212–13
richness 80–1
richness, calculating 80–1
variation in chromosome numbers 256
variation within 452–4
specific e98
specificity e62
specimen samples 49
spermatocytes 317
spermatogenesis 317
and oogenesis, comparison 319
spermatozoa 312
spindles 309
spliceosome 271
splicing 271
spontaneous mutations 290
stabilising selection 463
standard deviation e43–e44, e52
standard error e44–e45
formula e45
standard form e17
standard notation e17
statistical analysis e82, e83
statistics e42–e52
interpreting e42
sticky-end fragments
ligation 397
sticky-end restriction enzymes 395
stratified sampling 46–8
advantages 48
streams *see* freshwater
stromatolites 436
structural genes 278
student experiment e4, e53–e92 *see also* research
analysis of evidence criterion e55
conducting a e75–e79
ethical considerations e71–e72
four types of variables in e59
identifying a e57–e59
methodology e64, e72
one type of investigation e56
planning e64–e68
results e80–e86 *see also* results
risk assessment e69–e71, e77
summary e4, e53–e54
work schedule e64
student *t*-test *see t*-test
study group e119
study timetable e119
submetacentric chromosomes 255
submicroscopic representations e8, e10, e11–e12, e13, e14
subsoil 93, 96
subspecies 8
writing 9
succession 109, 191–200
sufficient e99
summary notes e120
sun 133
sunlight 135, 137, 146
Surtsey Island 198–9
surveys, conducting e56, e71
survival 453, 491
symbiont 160
symbiosis 15, 160
symbiotic relationships 13, 15
symbolic representations e8, e9, e10, e12, e14
sympatric speciation 481–2
syncytium 310
synonymous mutations 287
systematic errors e27, e29, e81, e83
systematics 445

t-test e47–e49, e52, e82
calculating, worked example e48
tables e35–e36, e41, e90, 49
Taq polymerase 405
Tasmanian devil 157–8, 491
TATA box 267
taxa 35, 36, 37, 436
taxon 5, 35
taxonomic group 436, 452, 485
true 37
taxonomy 5, 9, 445
teacher, as a resource e117
telocentric chromosomes 255
telomeres 266
telophase 309, 314, 315
temperature 182, 204–5, 210, 211
template strand 270
temporal isolation 476, 477
terrestrial biomes 130
terrestrial environments 91–9, 130
test cross 346
textbook e103
theoretical relationships, analysing e82–e83
thermophilic 405
third person narrative e90
thylacine 488, 491
thymine (T) 250
tolerance range 71
top soil 93
topic
Continuity of life on Earth (Unit 4, Topic 2) 431–2
Describing biodiversity (Unit 3, Topic 1) 3–4, 69
DNA, genes and the continuity of life (Unit 4, Topic 1) 247–8, 299, 325, 379
Ecosystem dynamics (Unit 3, Topic 2) 127–8
total foliage cover 76
total leaf cover 76
total percentage cover 77
traits 264, 338–9, 434, 454, 456, 460
transcription
explained 269, 273, 277
factors 269–70, 278
initiation 269
termination 270
transects 48
transfection 401
transfer RNA (tRNA) 253
transformation efficiency 410
transformed 401
transgene 386
transgenic animals 386–7
transgenic crops 387–8, 389, 390, 391
issues 392
transgenic organisms 386–7
translation
elongation 272
explained 271, 273, 277
initiation 267, 272
termination 272
translocation mutations 329
transporters *see* vectors
transposons 401
trees 136
trend lines e38
triggers, memory e118
triplet 265, 267
trophic cascade 166
trophic levels 140–1, 142, 146–7, 148
tropical rainforests 130
true-breeding 344
true value e27, e29, e33, e67, e82, e85
tumour-suppressor genes 281
turbidity tube 103
turgor 91
Turner syndrome 331
twin studies 283

uncertainties
calculating with e33
due to nature e33
and measurement e27–e34
uncertainty e29
calculating e29–e32
calculating, worked examples e30, e31
equation e30
and precision instruments e75, e76, e82
undirected network 84
units, SI *see* scientific notation (SI)
unrooted phylogenetic trees 38, 40
unweighted network 84
uracil (U) 250
'urkingdoms' 12
UV radiation 291

valid interpretation e85
validity e64–e65, e104–e105, 48
analysing e82–e83
variables
controlled e57, e59, e61, e65
defining e58–e59
dependent e36, e59, e61, e98

extraneous e82, e83
independent e35, e59, e61, e67, e98
measured e57, e59, e65
natural e67
and the research question rationale e63
types in student experiment e59
variety 9
writing 9
vectors 13, 163, 399
Vegetation Index 77
vegetative community 46
viability 453, 454, 457, 461
viable offspring 476
virulence 383
viruses 12
microevolution in 470

water
brackish 74
cycle 149–50
flow 104, 105
flow rate 105–6
flow rate, formula 106
and hydrosphere 131
oxygenation 104–5
oxygenation, measuring 105
speed 105, 106
supply 91, 92–3, 94
temperature 104–5, 182
temperature, measuring 105
waterways 106
Watson, James 259, 260
white soils 98
wild type 345
Wilkins, Maurice 259, 260
wind 91–2
Woese, Carl 12
wolphin 9
wordy language e90
working scientifically e3, e16–e52
summary e3, e16
writing *see also* report (for research investigation); scientific journal (your); scientific report (for student experiment)
fluently and concisely e88, e90
gene names 335
persuasive e90
scientific e89–e90
in a scientific genre e88–e89
time in the exam e122–e124

X-linked dominant inheritance 351–3, 360
ruling out 362
X-linked recessive inheritance 334, 349–50, 360
ruling out 362
X-rays 291
xenotransplantation 387

Y-linked inheritance 353, 361
ruling out 362
yellow crazy ants 87
Yellowstone National Park 14

zonation 194
zygote 300

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