BIOZONE

HSC | BIOLOGY

MODULES 5 - 8

BIOZONE

HSC | BIOLOGY
MODULES 5-8
Student Edition

BIOZONE Learning Media respectfully acknowledges the traditional custodians of the lands where we work and the places in which we live.

We pay our respects to ancestors and to all First Nations elders: past, present and emerging.

First edition 2022
ISBN 978-1-98-856655-9

First published 2022 by BIOZONE International Ltd

First printing

Printed by Greenridge Press (Australia) with paper manufactured using resources and methods that have a low environmental impact.

Acknowledgements

BIOZONE wishes to thank and acknowledge the team for their efforts and contributions to the production of this title.

Cover Photograph

Photo: Adobe Stock - davidcarbo

Green turtles (*Chelonia mydas*) migrate long distances between feeding grounds and the beaches from where they hatched. Classified as endangered, green turtles are threatened by overharvesting of their eggs, hunting of adults, entrapment in fishing gear, and loss of nesting beach sites.
https://www.worldwildlife.org/species/green-turtle

Disclaimer

Although every care has been taken, Aboriginal and Torres Strait Islander people should be aware that this title may contain images of deceased persons.

The external weblinks (URLs) referenced in this book were correct at the time of publishing. However, due to the dynamic nature of the internet, some addresses may have changed, or cease to exist. While BIOZONE regrets any inconvenience that this may cause readers, no responsibility for any such changes or unforeseeable errors can be accepted by BIOZONE.

BIOZONE Learning Media Australia

P.O. Box 2841, Burleigh BC,
QLD 4220, Australia

07 5535 4896
07 5508 2432
sales@biozone.com.au

www.BIOZONE.com.au

HSC | BIOLOGY MODULES 5-8

About the Authors

Kent Pryor *Author*

Kent has a BSc from Massey University, majoring in zoology and ecology and taught secondary school biology and chemistry for 9 years before joining BIOZONE as an author in 2009.

Jillian Mellanby *Editor*

Jill began her science career with a degree in biochemistry and, after a short spell in research labs, became a science teacher both in the UK and then New Zealand. She spent many years managing the Royal Society of New Zealand's academic publishing programme of eight science journals which allowed her to hone her project management and editorial skills. She was also a part of the Expert Advice writing team at the Royal Society of New Zealand, producing science pieces for a public audience. She joined the BIOZONE team in late 2021, as editor.

Sarah Gaze *Author*

Sarah has 16 years experience as a Science and Chemistry teacher, recently completing MEd. (1st class hons) with a focus on curriculum, science, and climate change education. She has a background in educational resource development, academic writing, and art. Sarah has recently joined the BIOZONE team, at the start of 2022.

Lissa Bainbridge-Smith *Author*

Lissa graduated with a Masters in Science (hons) from the University of Waikato. After graduation she worked in industry in a research and development capacity for eight years. Lissa joined BIOZONE in 2006 and is hands-on developing new curricula. Lissa has also taught science theory and practical skills to international and ESL students.

Contents

MODULE 5: Heredity

Chapter 1: Reproduction

Chapter 2: Cell Replication

Chapter 3: DNA and Polypeptide Synthesis

Chapter 4: Genetic Variation

Activity is marked: ☐ to be done; ☑ when completed ● Includes practical investigation

Contents

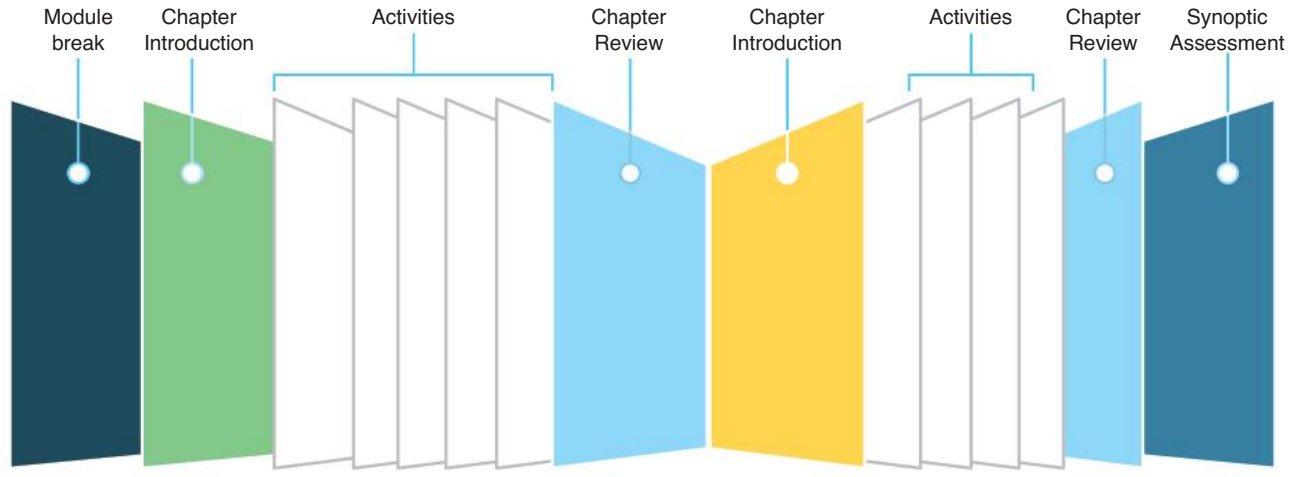
Activity is marked: ☐ to be done; ☑ when completed ● Includes practical investigation

Contents

Using This Worktext

HSC Biology: Modules 5 - 8 has been specifically written to meet the content and skills requirements of the NSW Stage 6 syllabus (Modules 5 - 8). The worktext follows the structure outlined in the Stage 6 syllabus, making it easy for you to know where you are in the course. Module breaks divide the content into sections (the modules) and summarise the student outcomes for each module. Each chapter has an introductory page, allowing you to see the key knowledge and skill requirements for each chapter. You can review and test your understanding, and prepare for assessments and exams, by carrying out the Chapter Review and Synoptic Assessment activities.

- A structure of a module is outlined below to help you identify the features within each module.



Chapter introduction

- Inquiry questions are identified.
- A checklist of key knowledge.
- A list of key terms.

Activity pages

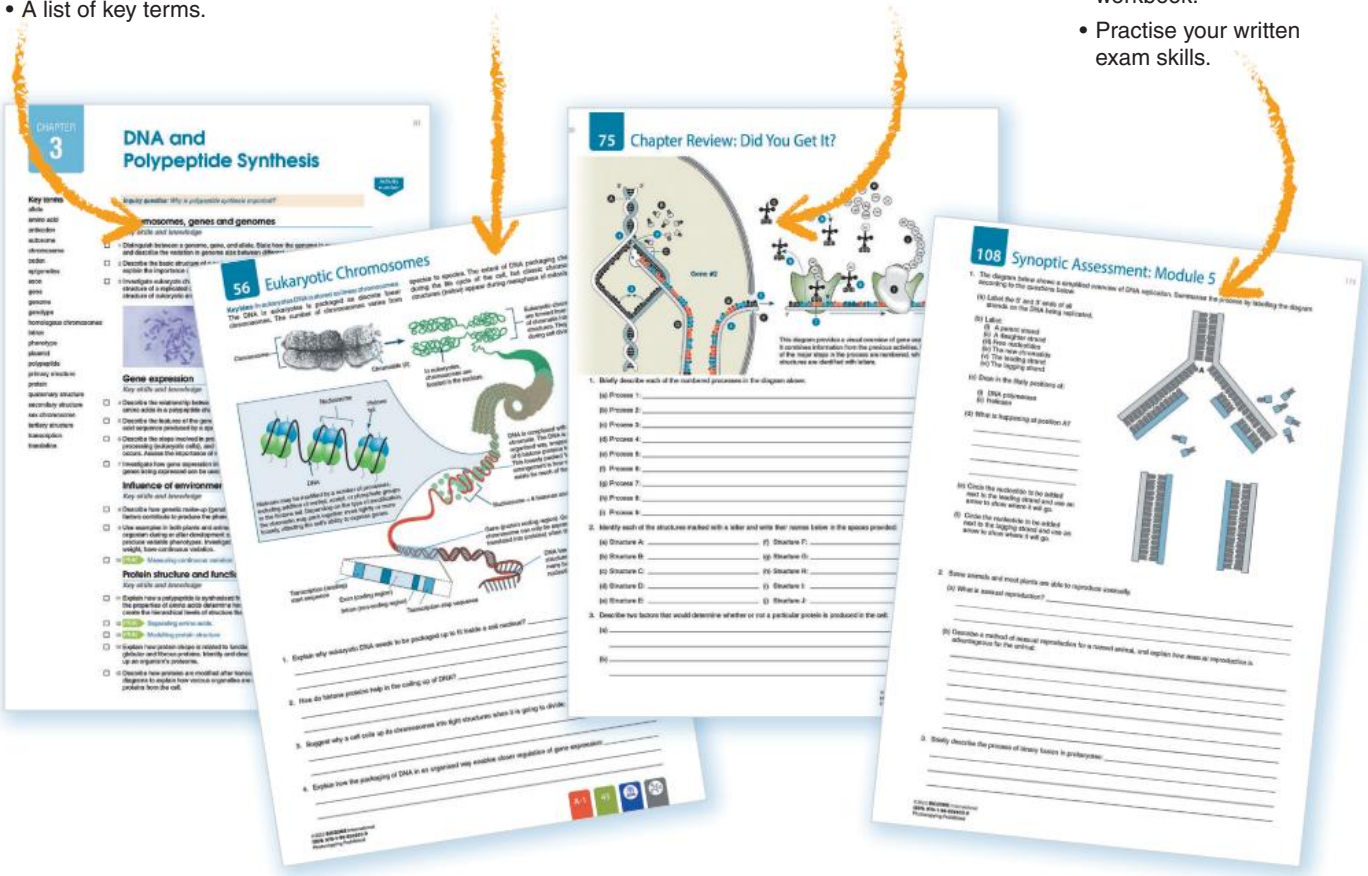
- Contain essential knowledge.
- Questions review the content of the page.

Chapter review

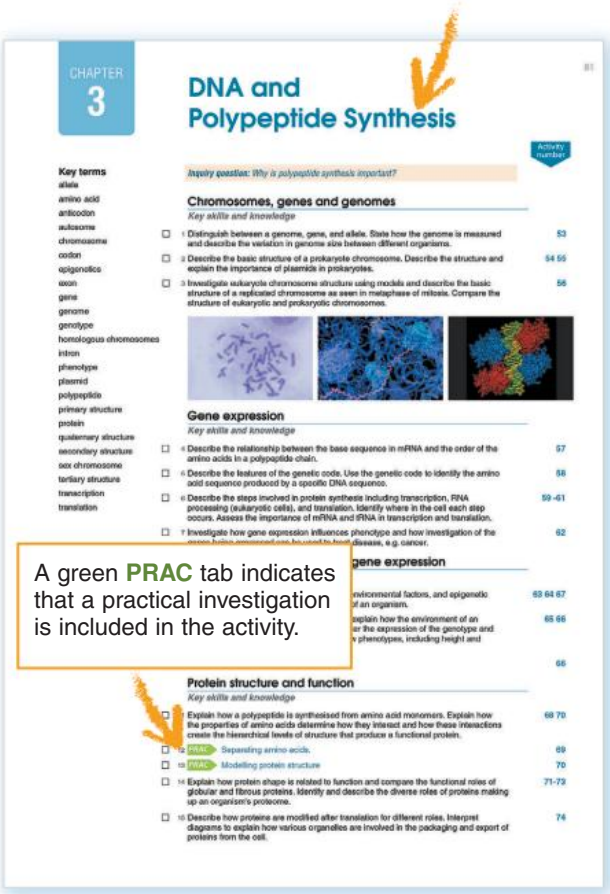
- Test your understanding of the chapter content.
- Develop your scientific literacy.

Synoptic assessment

- Synoptic assessments conclude the module of study covered in the workbook.
- Practise your written exam skills.

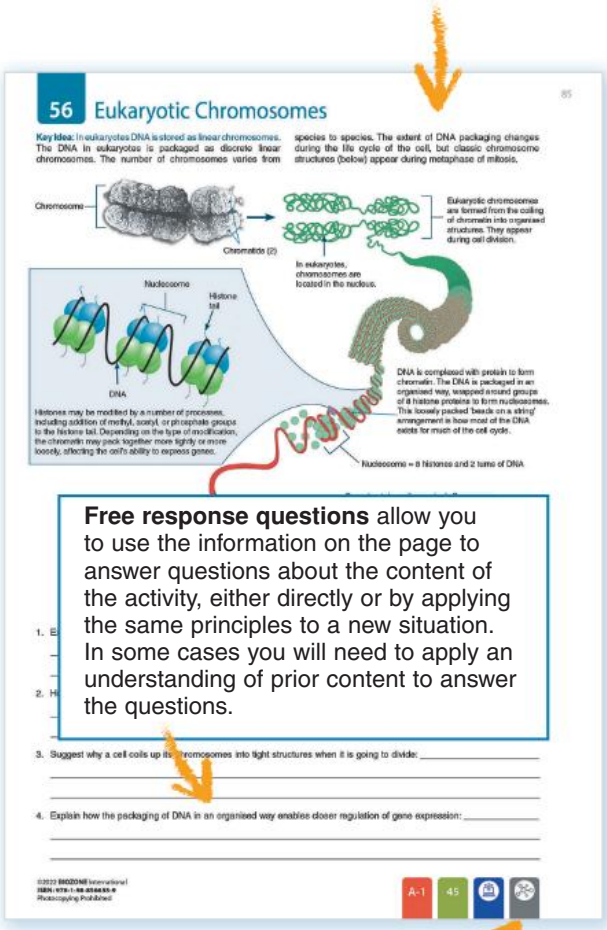


The **chapter introduction** identifies the inquiry question for the chapter. Keep this in mind as you work through the content, and try to relate your learning back to it. The chapter introduction also provides you with a summary of the key skills and knowledge requirements for the topic, written as a set of learning outcomes. Use the check boxes to identify and mark off the points as you complete them. A list of key terms for the chapter is provided to help you develop your scientific vocabulary.

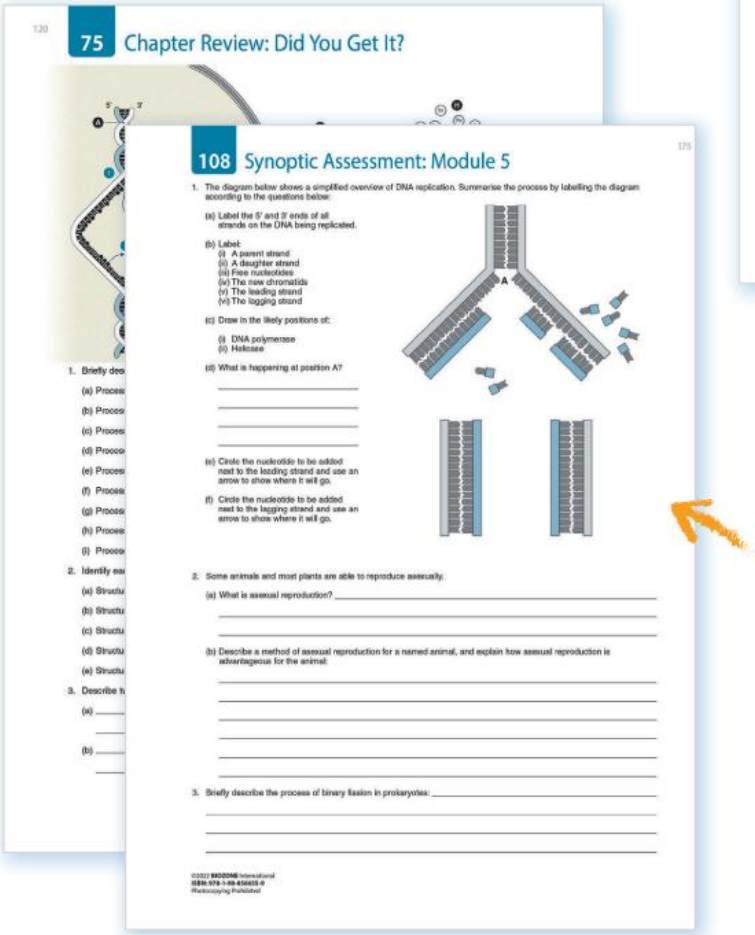


A green **PRAC** tab indicates that a practical investigation is included in the activity.

The activities form most of the workbook. The activity number is found at the top of the first page. Each activity has a short introduction with a key idea identifying the main message of the page. Lots of diagrams and photos are used to deliver the information to you.



Free response questions allow you to use the information on the page to answer questions about the content of the activity, either directly or by applying the same principles to a new situation. In some cases you will need to apply an understanding of prior content to answer the questions.



More information about the tabs can be found on page viii

Prepare for tests and exams by using the in-built assessment tasks in the worktext.

Chapter Review: Did You Get It? activities at the end of each chapter will test how well you know the content of the chapter, and are a great way to improve scientific understanding and vocabulary.

Synoptic Assessment activities at the end of each module are more formal testing moments. These are good activities for test and exam preparation, and provide practise in writing longer, exam type answers.

Using the Tab System

The tab system helps you identify important parts of the HSC Biology course (general capabilities, cross-curriculum priorities, and other curriculum learning areas). The tabs also allow you to see, at a glance, if online support is provided on BIOZONE's **Resource Hub**, and if there are content links with other activities. A summary of the icon tabs is provided below.

General capabilities

	**Critical & creative thinking**: Develop critical and creative thinking skills through asking questions, making predictions, engaging in practical and secondary-sourced investigations, and anlaysing and evaluating evidence.
	**Ethical understanding**: Apply ethical values and principles to your studies and investigations. Understand the implications of these to others and the environment, and that reasoning can assist in making ethical judgements.
	**Information & communication technology capability**: Use ICT to access information; collect, analyse, and represent data; model and interpret concepts and relationships; process information;and communicate ideas.
	**Intercultural understanding**: Appreciate and respect diverse cultures (yours and others) and understand how cultural perspectives have impacted the development, breadth, and diversity of scientific knowledge and applications.
	**Literacy**: Literacy is the ability to identify, understand, interpret, create, and communicate effectively using written, visual, oral, and digital formats. Apply these skills to communicate scientific concepts and findings.
	**Numeracy**: Numeracy involves recognising and understanding the role of mathematics in the world. Develop numeracy skills by measuring, recording, representing, and anlaysing data.
	**Personal & social capability**: Establish positive relationships, make responsible decisions, work effectively (alone and with others), and constructively handle challenging situations during your scientific endeavours.

Cross-curriculum priorities

	**Aboriginal & Torres Strait Islander histories & cultures**: The traditional knowledge and cultural practices of Aboriginal & Torres Strait Islander peoples provide insight into how the environment and natural world work. Traditional knowledge and Western scientific knowledge can be used together in a complementary way.
	**Asia & Australia's engagement with Asia**: The diverse environments of Australia and Asia provide opportunities to study interactions within and between the two environments, including how human activity influences the region, and the significance of these to the rest of the world.
	**Sustainability**: Sustainability is concerned with the ongoing capacity of the Earth to maintain all life. It provides contexts for exploring, investigating, and understanding the interrelatedness and sustainability of Earth's systems, including both natural and human-made environments.

Other learning across curriculum areas

	**Civics & citizenship**: Understand how civics, the understanding of Australian society, and citizenship can be applied to scientific ideas and technological advances.
	**Difference & diversity**: Australian society is diverse in terms of gender, race, and socio-economic circumstances. Working collaboratively provides opportunities to develop an appreciation of the values and ideas of others.
	**Work & enterprise**: Develop and use safe working practices. Identify risks and carry out hazard assessments when working in the laboratory or field.

Other tabs

	Grey hub tabs indicate that the activity is supported by content on BIOZONE's Resource Hub. See page ix for details about BIOZONE's Resource Hub.
7	Green tabs show connections to related activities and content elsewhere in the book.
A-1	Appendix 1: Glossary of key terms and their definitions.
A-2	Appendix 2: Equipment list for the practical investigations.

Using BIOZONE's Resource Hub

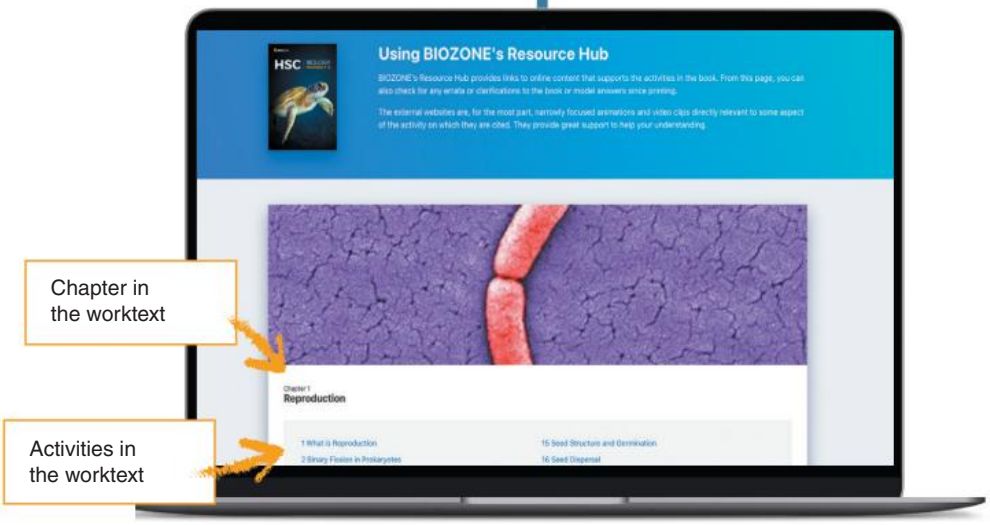
- **BIOZONE's Resource Hub** provides links to online content supporting the activities in the book. From this page, you can also check for any errata or clarifications to the book since printing.
- Many of these external websites are narrowly focused animations and video clips directly relevant to that part of the activity identified by the hub icon. There is also material for data exploration, source material for activities, and some fact sheets, as well as 3D models and spreadsheet models. The hub provides great support for your studies.

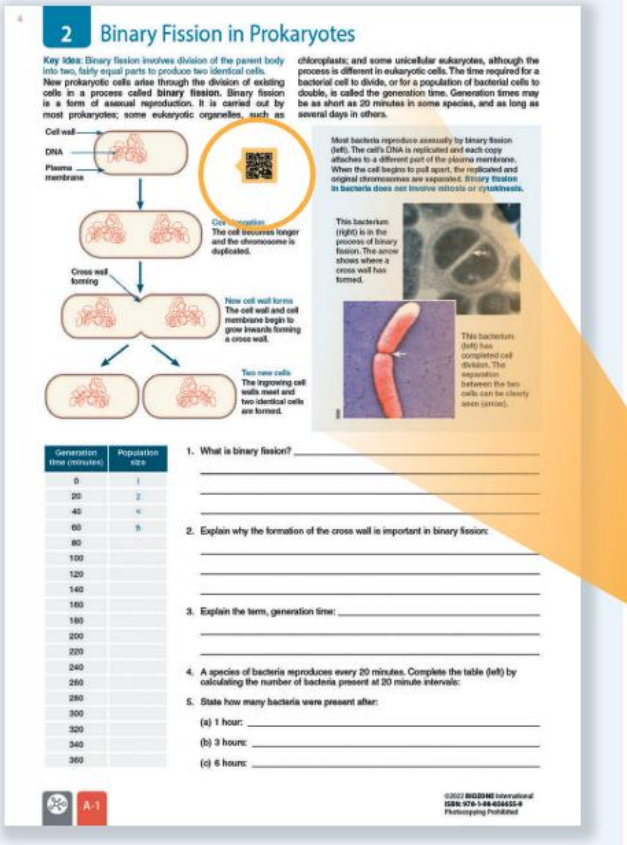
www.BIOZONEhub.com

Then enter the code in the text field

HSC12-1-6559

Or scan this QR code





Scan the **QR codes** on the activity pages. These link directly to informative and engaging 3D models. All models can be rotated and zoomed, and some contain informative annotations.

Practical Investigations

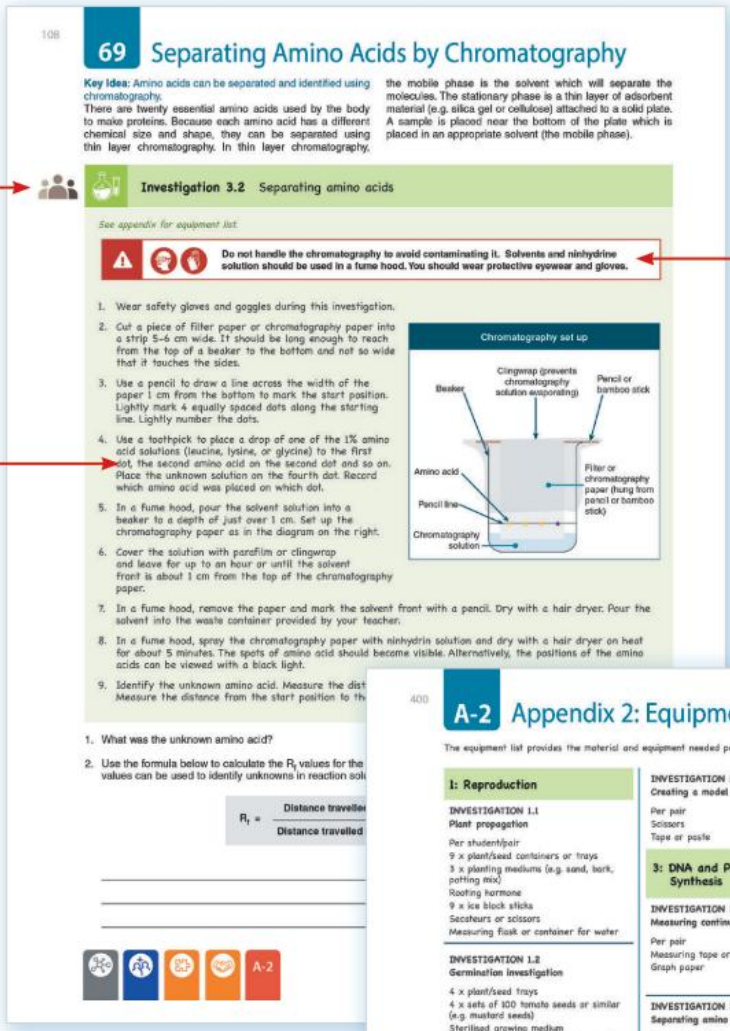
Carrying out practical investigations is an important component of the HSC Biology course. Hands-on investigations provide opportunities for you to develop good laboratory and investigative skills, and to work with others. Working in groups allows you to experience the benefits of collaboration. Scientific vocabulary is extended as you listen to the others' ideas, and share and discuss your own ideas. This worktext includes a number of simple practical investigations like the one shown below.

This icon shows if you need to work in pairs or groups.

Always follow safe laboratory practices. Any specific safety notes will be provided here. Your teacher may have more instructions.

Read all of the instructions **before** you begin so you are well prepared.

Equipment lists for each investigation are provided in **Appendix 2** at the back of the book.



108

69 Separating Amino Acids by Chromatography

Key idea: Amino acids can be separated and identified using chromatography.

There are twenty essential amino acids used by the body to make proteins. Because each amino acid has a different chemical size and shape, they can be separated using thin layer chromatography. In thin layer chromatography, the mobile phase is the solvent which will separate the molecules. The stationary phase is a thin layer of adsorbent material (e.g. silica gel or cellulose) attached to a solid plate. A sample is placed near the bottom of the plate which is placed in an appropriate solvent (the mobile phase).

Investigation 3.2 Separating amino acids

See appendix for equipment list.

Do not handle the chromatography to avoid contaminating it. Solvents and ninhydrine solution should be used in a fume hood. You should wear protective eyewear and gloves.

1. Wear safety gloves and goggles during this investigation.
2. Cut a piece of filter paper or chromatography paper into a strip 5–6 cm wide. It should be long enough to reach from the top of a beaker to the bottom and not so wide that it touches the sides.
3. Use a pencil to draw a line across the width of the paper 1 cm from the bottom to mark the start position. Lightly mark 4 equally spaced dots along the starting line. Lightly number the dots.
4. Use a toothpick to place a drop of one of the 1% amino acid solutions (leucine, lysine, or glycine) to the first dot, the second amino acid on the second dot and so on. Place the unknown solution on the fourth dot. Record which amino acid was placed on which dot.
5. In a fume hood, pour the solvent solution into a beaker to a depth of just over 1 cm. Set up the chromatography paper as in the diagram on the right.
6. Cover the solution with parafilm or clingwrap and leave for up to an hour or until the solvent front is about 1 cm from the top of the chromatography paper.
7. In a fume hood, remove the paper and mark the solvent front with a pencil. Dry with a hair dryer. Pour the solvent into the waste container provided by your teacher.
8. In a fume hood, spray the chromatography paper with ninhydrin solution and dry with a hair dryer on heat for about 5 minutes. The spots of amino acid should become visible. Alternatively, the positions of the amino acids can be viewed with a black light.
9. Identify the unknown amino acid. Measure the dist... Measure the distance from the start position to th...

Chromatography set up

1. What was the unknown amino acid?
2. Use the formula below to calculate the R_f values for the ... values can be used to identify unknowns in reaction sol...

$$R_f = \frac{\text{Distance travelled}}{\text{Distance travelled}}$$

A-2

400

A-2 Appendix 2: Equipment List

The equipment list provides the material and equipment needed per student, pair, or group.

1: Reproduction

INVESTIGATION 1.1
Plant propagation

Per student/pair
9 x plant/seed containers or trays
3 x planting mediums (e.g. sand, bark, potting mix)
Rooting hormone
9 x ice block sticks
Secateurs or scissors
Measuring flask or container for water

INVESTIGATION 1.2
Germination investigation

4 x plant/seed trays
4 x sets of 100 tomato seeds or similar (e.g. mustard seeds)
Sterilised growing medium
Measuring flask or container for water

2: Cell Replication

INVESTIGATION 2.1
Modelling mitosis

String
4 x pipe-cleaners (2 colors) cut in half
A3 sheet of paper
Marker

INVESTIGATION 2.2
Modelling meiosis using ice block sticks

Per student/pair
6 x 500 mL beakers
Balance and equipment to weigh sugar
Table sugar or lab sucrose
Potato
Cork borer or scalpel
Paper towels
Marker pen

INVESTIGATION 2.3
Extracting DNA

Per pair
5 – 6 strawberries
1 large zip-lock bag
100 mL water
5 mL detergent
pinch of salt
1 x filter paper
1 x glass filter funnel
1 x 250 mL glass beaker
1 x glass rod
~100 mL ethanol (for rinsing)
2 x centrifuge tubes
Centrifuge

INVESTIGATION 2.4
Creating a model of a DNA molecule

Per pair
Scissors
Tape or paste

3: DNA and Polypeptide Synthesis

INVESTIGATION 3.1
Measuring continuous variation

Per pair
Measuring tape or scales
Graph paper

INVESTIGATION 3.2
Separating amino acids

Per student/pair
Filter paper or chromatography paper
Pencil
Clingwrap or parafilm
Scissors
1% amino acid solutions (leucine, lysine, glycine).
Chromatography solution (butan-2-ol, glacial ethanoic acid, water in ratio 6:1.5:2)
Ninhydrin spray or black light
Nitrile gloves

INVESTIGATION 3.3
Modelling protein structure

Per student/pair/group
Pipe cleaners (2 white, 2 pink, 2 purple, 4 blue)
Sticky tape
2 x binder clips or paper clips

4: Genetic Variation

INVESTIGATION 4.1
Measuring continuous variation

Computer with spreadsheeting programme e.g. Excel.

6: Mutation

INVESTIGATION 6.1
Investigating natural selection

Per student
Computer
Spreadsheet application (e.g. Excel)

INVESTIGATION 6.2
Modelling genetic drift

Per student
Computer
Spreadsheet application (e.g. Excel)

9: Causes of Infectious Disease

INVESTIGATION 9.1
Investigating microbial contamination in food samples

Per student or group
Food sample
Agar plates
Inoculation loops
Bunsen burner
Sterilising alcohol
Test tubes
Glass rods
Distilled water
Tape
Marker pens
Incubator

12: Prevention, Treatment and Control

INVESTIGATION 12.1
Investigating the effectiveness of handwashing

Per class
Warm water
Soap
Hand sanitiser

Per individual
1 x nutrient agar plates
Marker pen
Paper towels
Incubator (if using)

INVESTIGATION 12.2
Modelling disease outbreak and spread

Per pair
Computer
Spreadsheet application (e.g. Excel)

Support for Depth Studies

During your second year of the HSC Biology course you will carry out another depth study.

There are too many options for us to cover in this worktext, but we have provided some helpful information to help you make your depth study a success! Chapter 18 of this worktext provides you with information for choosing what type of depth study you want to do. It provides guidance on planning and carrying out the study, and also some tips on how to share your findings with others. Refer to these pages often as you plan and work on your depth study. Your teacher will also help with your choice, and will provide more information about how you can meet the syllabus requirements for the depth study.

Chapter 18 provides support to help you plan and carry out your depth study

Choosing your depth study

- What types of studies, projects, or investigations can be used for a depth study?
- What type of study is most appropriate for the topic I want to study?
- What are the differences between a primary practical investigation and a secondary-sourced investigation?

Sharing your findings

- What communication style is best for my study?
- What style of communication do I prefer or feel confident with?
- How should I structure the way I deliver my findings?

Planning your depth study

- What does the planning process look like?
- What do I need to think about when planning my depth study?
- What do I want to find out from my study?
- What type of data should I collect, and how will I analyse it?
- What equipment will I need?
- How long will it take?

Referencing

- How do I acknowledge online resources?

Answering Exam Questions

- Exams require you to demonstrate your understanding of a particular concept by providing a written paragraph or essay.
- Open answer questions (meaning there is no definitive answer) are designed so that you can demonstrate your level of understanding. The question may give you some guidance as to what you should include in your answer, such as defining certain terms or providing specific examples.
- In order to gain the highest possible mark in these questions, you need to organise your answer in a clear and logical way so that the examiner can easily see how you have demonstrated your understanding of the topic.
- The difference between you obtaining a low, mid, or high grade depends on how well you demonstrate your understanding of a concept.

• Defining, drawing, annotating, or giving a description demonstrates a basic understanding of the material.

• Explaining how a process works, why it works, and how changes to it may affect an outcome, shows a deeper understanding of how the system works in that situation.

• Linking biological ideas, comparing and contrasting, analysing, or justifying ideas shows both a deep understanding, and an ability to translate that understanding to a new situation.

- The following example shows how an answer can be built up from a simple definition, through explanation, to comparisons and linking of ideas.

The human immune system detects pathogens and abnormal cells and launches an attack against them to prevent them doing harm.
Outline the structure of the human immune system, and explain why a tiered defence system is an advantage when the body encounters a pathogen.

The human immune system consists of three lines of defence. The combination of physical, chemical, and physiological (adaptive) responses provides a multi-pronged immune response to pathogens. If one level of defence fails, there are other defences to minimise the opportunity for a pathogen to cause infection. The immune system falls into two categories - the innate response and the adaptive response.

The innate immune system provides a non-specific response. It reacts and behaves the same way every time, regardless of the pathogen encountered. The first two lines of defence are innate responses. The skin is a physical barrier to pathogen entry, and chemical secretions inhibit microbial growth. Tears, mucus and saliva wash microbes away.
Inside the body, neutrophils and macrophages engulf and break down pathogens, the complement system is important in this response by activating immune system cells. Other white blood cells release antimicrobial substances to destroy pathogens. The inflammatory response helps contain and destroy the pathogen,and begins healing damaged tissue.

The adaptive immune response is a targeted response. A specific immune response is launched for each different type of pathogen encountered. Specialised white blood cells (B and T lymphocytes) are key to this response. Antigen presenting cells present antigens to T helper cells. The T helper cells then send chemical signals to stimulate T killer cells (these destroy the pathogen). T helper cells also stimulate the production of antibody producing B cells. Each type of B cell recognises one specific antigen and produces the correct antibody to destroy it. Memory B cells are also produced, so if the pathogen is encountered again a faster immune response is launched.

The advantage of a tiered defence system is explained.

Overview of the innate immune system is provided.

Overview of the adaptive immune system is provided.

The two categories of the immune system (innate and adaptive) are introduced.

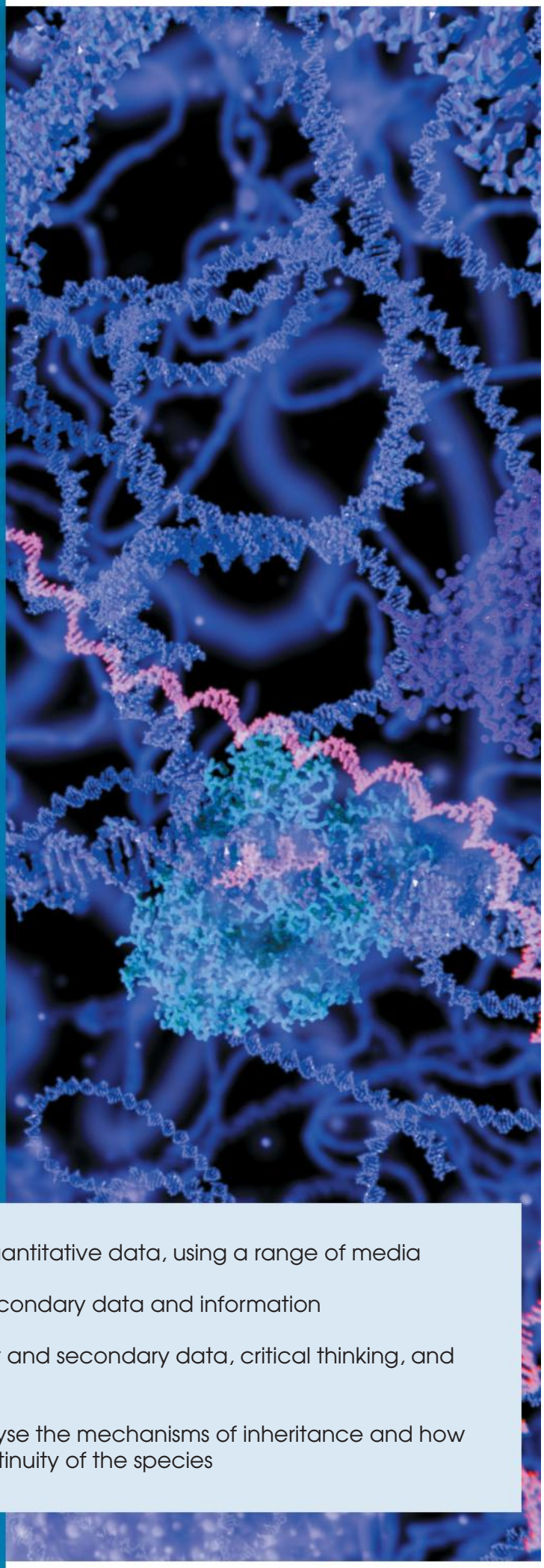
Specific details about the first two lines of defence (innate responses) are provided.

Specific details about the third line of defence (adaptive responses) are provided.

MODULE

05

Heredity



Student outcomes:

- Select and process qualitative and quantitative data, using a range of media
- Analyse and evaluate primary and secondary data and information
- Solve scientific problems using primary and secondary data, critical thinking, and scientific processes
- Explain the structure of DNA and analyse the mechanisms of inheritance and how processes of reproduction ensure continuity of the species

CHAPTER 1

Reproduction

Key terms

angiosperm
asexual reproduction
binary fission
budding
diploid
egg
fertilisation
fragmentation
gamete
gametophyte
germination
grafting
gymnosperm
haploid
micropropagation
oestrogen
oogenesis
pollination
seed
sexual reproduction
sperm
spermatogenesis
spore
sporophyte
testosterone
zygote

Inquiry question: *How does reproduction ensure the continuity of a species?*

Activity number

Mechanisms of reproduction

Key skills and knowledge

- ☐ 1 Explain the purpose of reproduction, and investigate the difference between sexual and asexual reproduction. Understand that asexual reproduction produces offspring identical to the parent and that sexual reproduction produces variation between the parents and offspring. — **1**
- ☐ 2 Describe asexual reproduction by fission in bacteria and unicellular eukaryotes. — **2 4**
- ☐ 3 Explain the mechanism of asexual reproduction by spores, fragmentation (e.g. bread mould), and budding (e.g. yeast) in fungi. Appreciate the complexity of fungal life cycles with many species carrying out both sexual and asexual reproduction (e.g. bread mould and yeast). — **3**
- ☐ 4 Describe asexual reproduction by budding and fragmentation in simple multicellular eukaryotes (e.g. *Hydra* and planarians). — **5**
- ☐ 5 Describe the diversity of mechanisms for asexual reproduction by vegetative propagation in plants. Include reference to natural vegetative structures such as bulbs, rhizomes, and tubers. — **6**
- ☐ 6 PRAC Investigate the effect of different growing mediums on the propagation of plant cuttings. — **7**
- ☐ 7 Investigate and describe the different mechanisms of sexual reproduction in plants, including ferns, gymnosperms, and angiosperms. Explain the uses of wind and insect pollination in plants. — **8-16**
- ☐ 8 PRAC Investigate the effect of water on the germination of plant seeds. — **15**
- ☐ 9 Describe animal reproductive strategies. Explain the advantages and disadvantages of external and internal fertilisation and the reasons for differences in energy allocation in reproductive effort. — **17-21**



Reproduction in mammals

Key skills and knowledge

- ☐ 10 Examine the differences in production of gametes in males and females. Understand that each gamete is haploid and unification of the gametes produces a zygote which is diploid. — **1 22 23**
- ☐ 11 Analyse the hormonal mechanisms that regulate the menstrual cycle and maintain pregnancy. — **25-26 29**
- ☐ 12 Explain the processes of fertilisation, implantation, and the early development of the embryo. Explain the feedback loops involved in the birth process and lactation. — **27-30**

Manipulating plant and animal reproduction

Key skills and knowledge

- ☐ 13 Use the examples of embryo splitting and artificial insemination to explain the manipulation of reproduction in livestock. Explain the use of grafting and micropropagation to propagate infertile crops (e.g. bananas). — **31-34**

1 What is Reproduction?

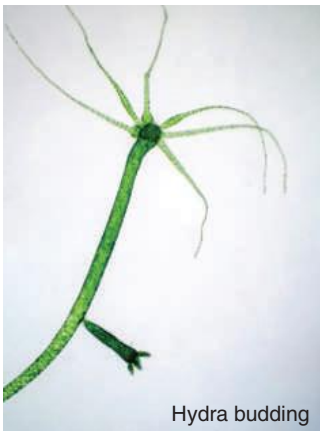
Key Idea: Reproduction is the production of offspring in order to continue a genetic lineage.

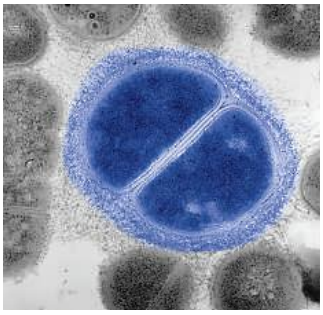
Reproduction is the production of new life that will carry on the genetic lineage and ensure the continuity of a species. Without it, an organism's genetic lineage is lost. There are two types of reproduction, asexual and sexual. **Asexual reproduction** produces offspring that are genetically identical to the parent. **Sexual reproduction** combines half the genetic material from each of two parents to produce offspring that are genetically distinct. Both types of reproduction have their advantages and disadvantages. Asexual reproduction is the primary form of reproduction in prokaryotes. It also occurs in eukaryotes, including plants, animals, and fungi. Sexual reproduction is the primary form of reproduction in many plants and in larger animals, including the vertebrates, and results in genetic variability.

Sexual vs asexual reproduction


Snails mating

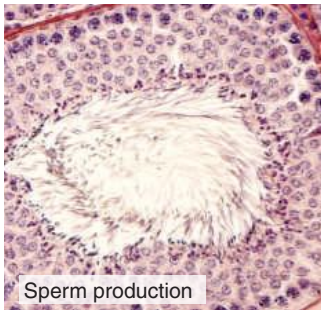
Sexual reproduction	Asexual reproduction
• Passes genetic information to the next generation • Production of offspring • Produces variation in offspring • Requires two parents • Offspring genetically related to both parents, but different from either • Relatively long time taken • E.g. pollination, fertilisation	• Ability to quickly take advantage of favourable environmental conditions • One parent required • Offspring genetically identical to parent • Passes genetic information to the next generation • Production of offspring • Relatively short time taken • E.g. budding, cloning


Hydra budding

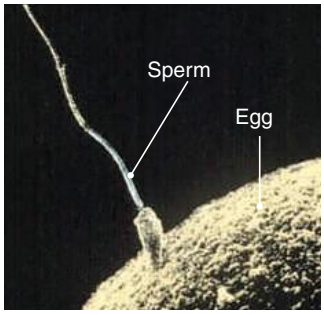


Prokaryotes, including bacteria, primarily reproduce by asexual reproduction, via binary fission.

Most plants can reproduce asexually by bulbs (above), corms, tubers, putting out under- or over-ground stems, or cuttings.


Sperm production

Sexual reproduction requires the production of **gametes**. These are the egg (female) and the sperm (male). Each gamete carries half the genetic compliment.



Unification of the gametes, called **fertilisation**, restores the full genetic complement and produces the first cell of the new individual, called the **zygote**.

1. What is the purpose of reproduction? _____

_____

2. What is the difference in the genetic relationship of an offspring to its parent(s) in asexual reproduction, compared to sexual reproduction?

_____

_____

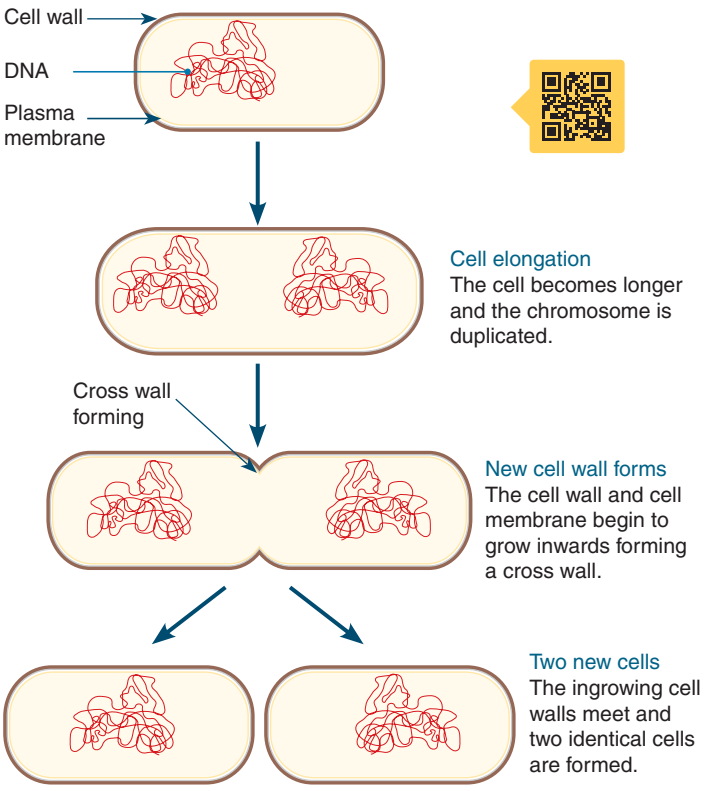
3. In the space below, produce a Venn diagram comparing and contrasting asexual and sexual reproduction:

ISBN: 978-1-98-856655-9

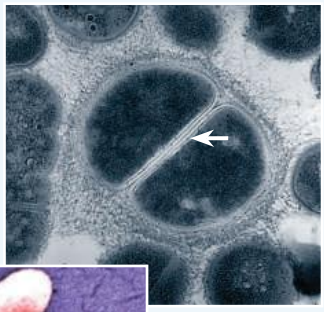
2 Binary Fission in Prokaryotes

Key Idea: Binary fission involves division of the parent body into two, fairly equal parts to produce two identical cells.

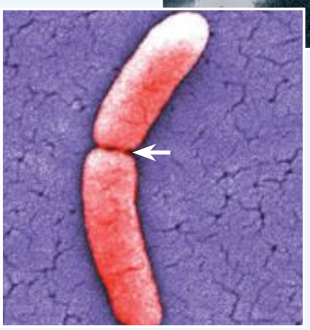
New prokaryotic cells arise through the division of existing cells in a process called **binary fission**. Binary fission is a form of asexual reproduction. It is carried out by most prokaryotes; some eukaryotic organelles, such as chloroplasts; and some unicellular eukaryotes, although the process is different in eukaryotic cells. The time required for a bacterial cell to divide, or for a population of bacterial cells to double, is called the generation time. Generation times may be as short as 20 minutes in some species, and as long as several days in others.



Most bacteria reproduce asexually by binary fission (left). The cell's DNA is replicated and each copy attaches to a different part of the plasma membrane. When the cell begins to pull apart, the replicated and original chromosomes are separated. **Binary fission in bacteria does not involve mitosis or cytokinesis**.



This bacterium (right) is in the process of binary fission. The arrow shows where a cross wall has formed.



CDC

This bacterium (left) has completed cell division. The separation between the two cells can be clearly seen (arrow).

Generation time (minutes)	Population size
0	1
20	2
40	4
60	8
80	
100	
120	
140	
160	
180	
200	
220	
240	
260	
280	
300	
320	
340	
360	

1. What is binary fission? _____

2. Explain why the formation of the cross wall is important in binary fission:

3. Explain the term, generation time: _____

4. A species of bacteria reproduces every 20 minutes. Complete the table (left) by calculating the number of bacteria present at 20 minute intervals:

5. State how many bacteria were present after:

   (a) 1 hour: _____

   (b) 3 hours: _____

   (c) 6 hours: _____

ISBN: 978-1-98-856655-9

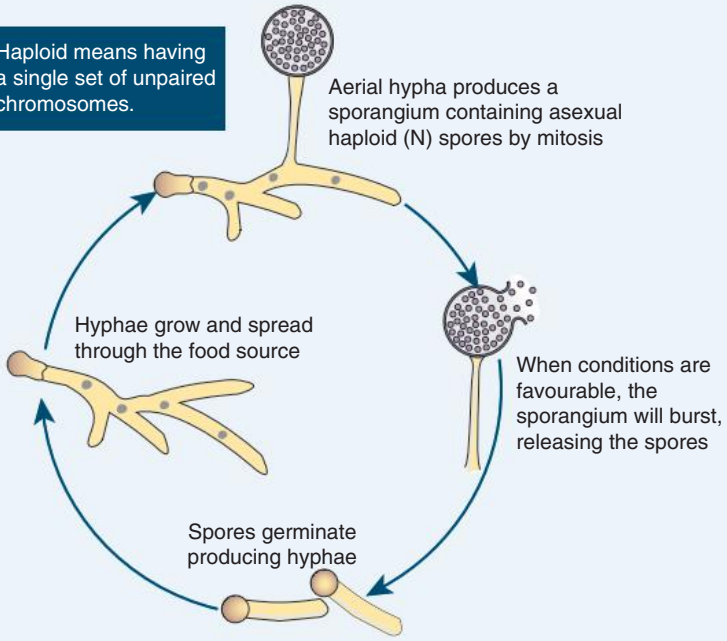
3 Reproduction in Fungi

Key Idea: Spores are the reproductive units produced for dispersal. Spores are usually unicellular and haploid.

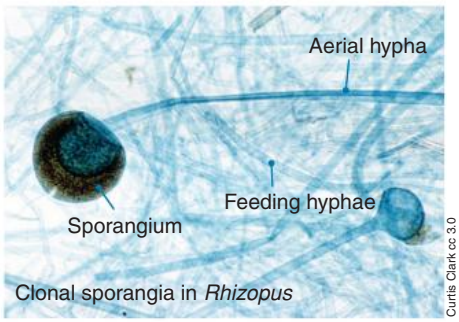
Many organisms, although not animals, produce spores as part of their normal reproductive life cycle. A spore is a unicellular, usually haploid, reproductive unit. It is a means of dispersal, reproduction and, in some cases, survival. Asexual reproduction by spores is common in fungi and some, including bread mould, rarely have a sexual phase in the life cycle at all. Huge numbers of spores may be produced, enabling rapid growth when food sources are available.

Asexual reproduction in bread mould, *Rhizopus*

Haploid means having a single set of unpaired chromosomes.



In *Rhizopus*, asexual reproduction by haploid spores is the main method of reproduction. In asexual reproduction, haploid hyphae produce upright sporangia, which release numerous haploid spores. These spores are dispersed widely and each germinates to form a new fungal body.



Curtis Clark cc 3.0

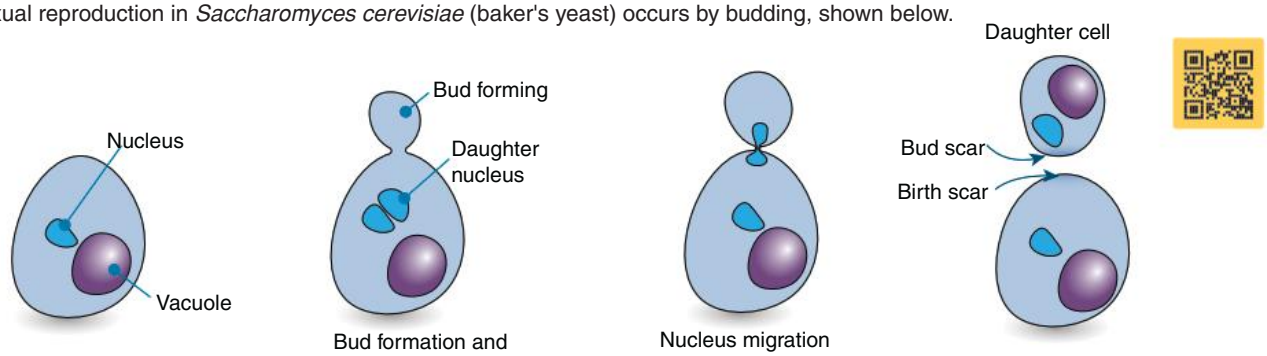
The asexual life cycle of bread mould involves the release of vegetative spores from sporangia. It can also grow from hyphae separated from another (parent) fungus, called fragmentation.

Kalyan Varma CC 3.0

All fungi disperse by spores, which are produced during both sexual and asexual reproduction. Commonly, a fruiting body, such as the above puffball, is produced in which the spores either develop or are shed. Spores are easily caught by the wind and spread over large distances.

Budding in yeast

Asexual reproduction in *Saccharomyces cerevisiae* (baker's yeast) occurs by budding, shown below.



1. What is a spore? _____

_____

2. What is the role of spores in the asexual reproduction of fungi? _____

_____

3. How are asexual spores produced in *Rhizopus*? _____

4. How is budding different from the production of spores? _____

_____

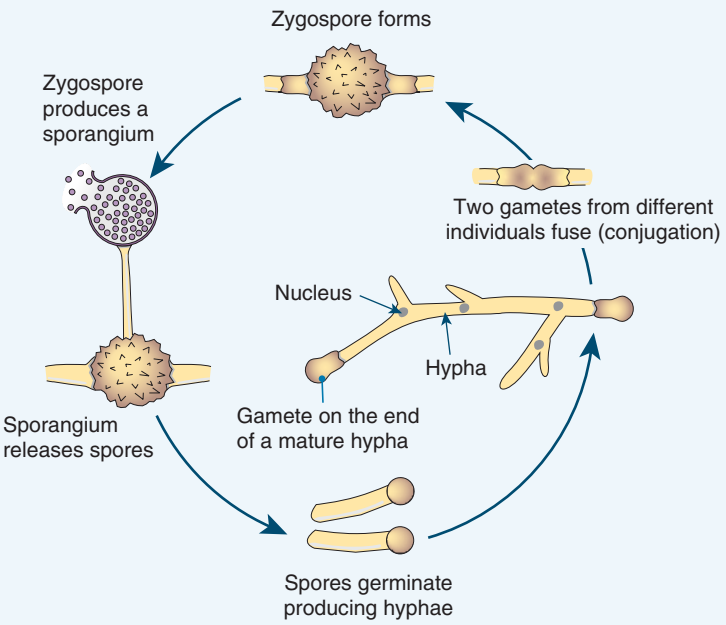
_____

ISBN: 978-1-98-856655-9

Sexual reproduction in fungi

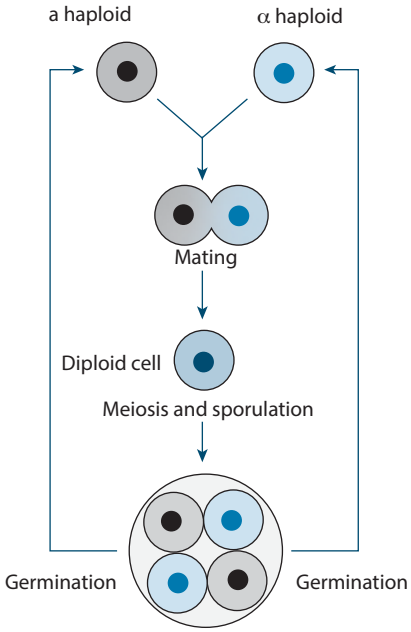
Fungi also carry out sexual reproduction, although it is usually much rarer than asexual reproduction. The process differs, depending on the species of fungus.

Sexual reproduction in bread mould, *Rhizopus*



Sexual reproduction in *Rhizopus* fungi occurs when two compatible mycelium fuse. The cells fuse to form diploid cells. A zygospore forms, containing many haploid spores formed by meiotic cell division. The spores are released and can form new mycelium.

Sexual reproduction in yeast



Saccharomyces cerevisiae also carries out sexual reproduction. It has two haploid mating types, a and α, with a single set of chromosomes. Each type can undergo asexual budding. The two cells can also fuse to form a diploid cell (2 paired sets of chromosomes) which can also bud asexually. This grows and, under starvation, begins meiosis, producing four haploid spores. These germinate and undergo asexual budding.

5. How do spores form in a zygospore? _____

6. Compare and contrast sexual reproduction in *Rhizopus* and yeast: _____

_____

_____

_____

7. Explain how reproduction in fungi maintains the continuity of the species: _____

_____

_____

_____

8. Use the space below to combine the asexual and sexual reproductive processes in yeast into one diagram, showing yeast's reproductive cycle:

ISBN: 978-1-98-856655-9

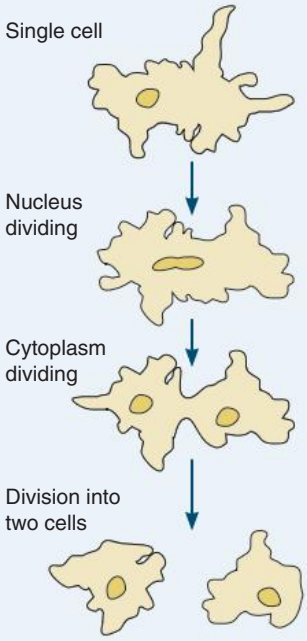
4 Asexual Reproduction in Protists

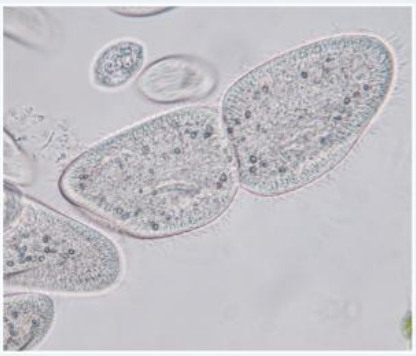
Key Idea: Protists can reproduce by binary fission or budding.

Protists are a diverse group of single celled eukaryotes. They include organisms such as amoeba, paramecium, and the slime moulds. Many of the different groups are not closely related. However, most reproduce by the simple methods of binary fission, or budding.

Binary fission in protists

- Unicellular eukaryotes can reproduce asexually by splitting in two. This process involves mitosis (replication of the DNA) and cytokinesis (division of the cell cytoplasm), and is not to be confused with the binary fission of bacteria, which does not involve mitosis and cytokinesis. Fission is shown (right) for an *Amoeba*.
- Note that some multi-nucleated amoebae and some life cycle stages of parasitic protozoa, such as the malarial parasite *Plasmodium*, undergo **multiple fission**. The nucleus divides repeatedly before the final division of the cytoplasm to produce many new cells. Repeated cycles of multiple fission rapidly produce large numbers of offspring.

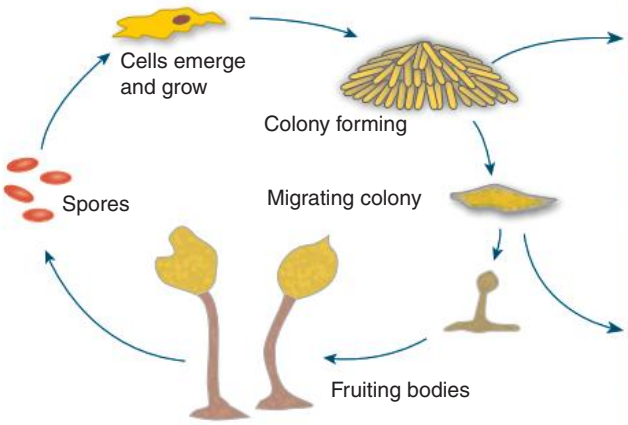




Binary fission is common in protists. In the image above, a *Paramecium* splits to form two new cells.

Asexual reproduction in cellular slime moulds

- Due to their diverse nature, protists have many different reproductive methods. The cellular slime mould *Dictyostelium* normally lives as a single celled amoeba-like organism.
- However, when food becomes scarce, many thousands of cells will group together to form a structure called a slug. This may travel to a new location before forming a fruiting body, which produces and releases spores that will develop into new individuals.



1. What is the purpose of binary fission in protists? _____

2. How is binary fission different from multiple fission? _____

3. What is the purpose of colony formation in cellular slime moulds? _____

ISBN: 978-1-98-856655-9

5 Fragmentation and Budding in Simple Animals

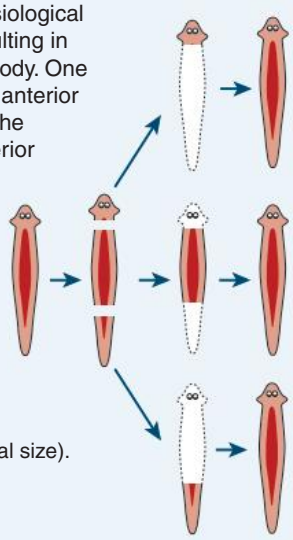
Key Idea: Animals with relatively simple body plans are capable of reproducing from fragmentation or budding.

Cutting complex animals such as mammals or birds into three or four equal sized pieces would mean instant death. But for simple animals, such as flat worms or cnidarians (includes jellyfish and anemones), it can lead to the production of new individuals as each fragment regrows. Each new individual is genetically identical; they are clones. Cnidarians also reproduce by budding. On the body of the animal a bud will form that will grow into a new clone. This will become an independent individual and may remain attached or separated.

Fragmentation

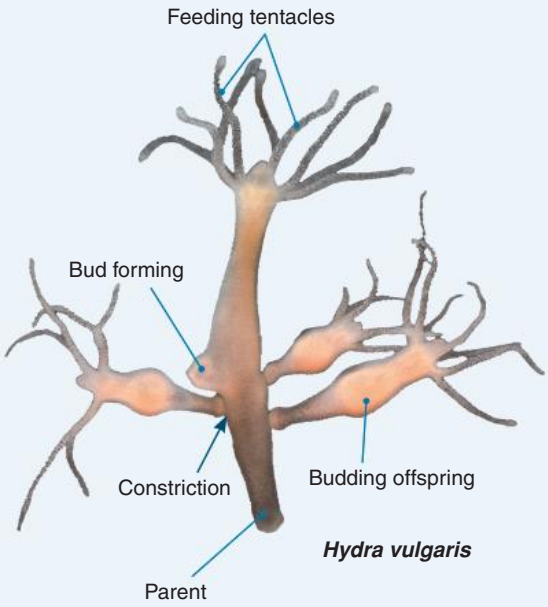
- Some cnidarians, sponges, and flatworms can reproduce by fragmentation. In this process, the organism spontaneously divides into fragments. Each fragment develops into a mature, fully grown individual, identical to the original organism.

- In flatworms, a physiological gradient exists, resulting in polarisation of the body. One pole represents the anterior (head) region, and the other pole the posterior (tail) region. This ensures that each new organism develops normally, i.e. they don't have two heads.
- Planarians (above) can regenerate from very small fragments (1/279th of the original size).



Budding

- Sponges and most cnidarians (e.g. Hydra) can reproduce by budding. A small part of the parent body separates from the rest and develops into a new individual, which is smaller than the parent. This new individual may remain attached as part of the colony, or the bud may constrict at its point of attachment and be released as an independent organism.
- The photo below shows *Hydra* budding. The new individuals are budding from the main body of the parent animal. The photograph shows the bulge and constriction where each new offspring will separate to form an independent individual.



1. What is the advantage of fragmentation to a flatworm? _____

2. All individuals produced by budding or fragmentation are clones. Explain why this might leave a population vulnerable if environmental conditions rapidly changed:

3. Research fragmentation and list any animals, or groups of animals, that appear to be able to reproduce from fragmentation:

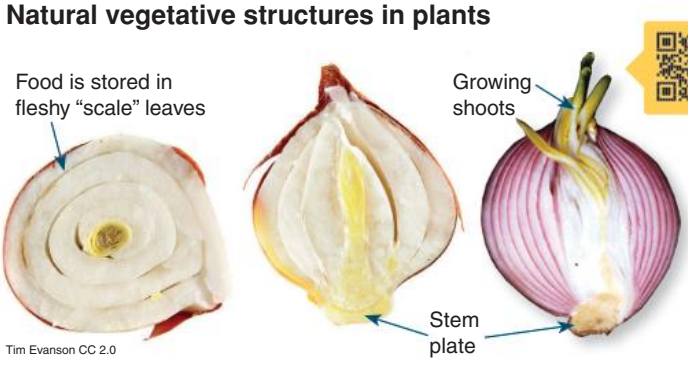
ISBN: 978-1-98-856655-9

6 Asexual Reproduction in Plants

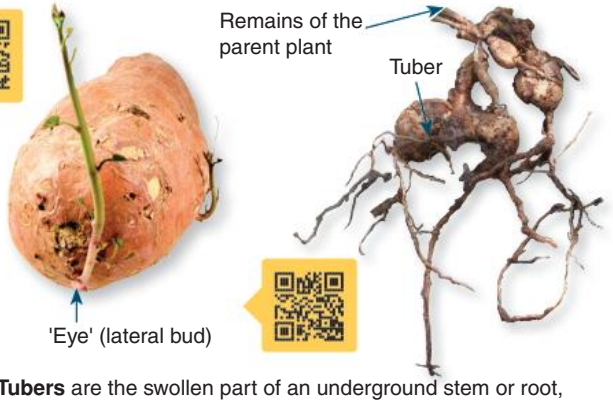
Key Idea: Plant propagation can quickly produce large numbers of genetically identical individuals.

Many flowering plants reproduce asexually by **vegetative propagation.** This is the process by which new plants arise from vegetative tissues of the parent plant. This ability is the result of the **totipotency** exhibited by plant cells. Vegetative propagation allows plants to spread rapidly in favourable conditions, avoiding the high energy cost of producing flowers, pollen, seeds, or fruits. Humans exploit the vegetative abilities of plants widely, so that many crop strains today are hardly ever grown from seed. Vegetative propagation enables successful varieties to be propagated indefinitely.

Natural vegetative structures in plants

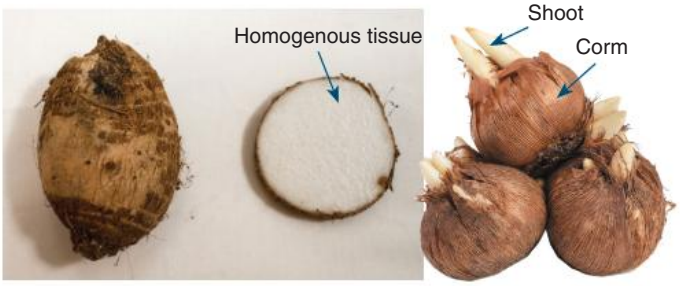


A **bulb** is really just a typical shoot compressed into a shortened form. Fleshy storage leaves are attached to a stem plate and form concentric circles around the growing tip. New roots form from the lower part of the stem.

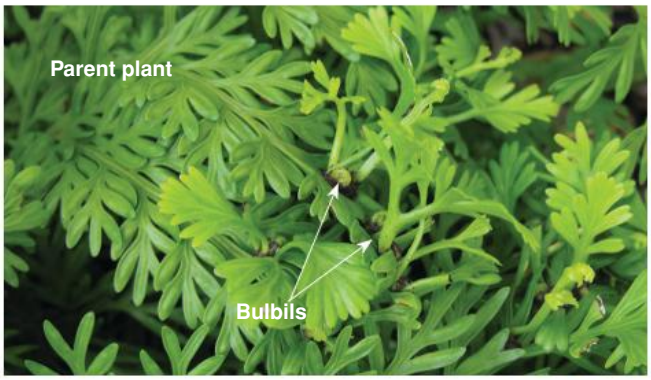


Tubers are the swollen part of an underground stem or root, usually modified for storing food. The potato (above, left) is a stem tuber, as indicated by the presence of terminal and lateral buds. The dahlia (above, right) is a root tuber.

In **rhizomes**, food is stored in the horizontal, underground stem. Rhizomes tend to be thick, fleshy or woody, and bear nodes with scale or foliage leaves and buds. Growth occurs at the buds on the ends of the rhizome or nearby nodes. Ginger, turmeric, irises, and lily-of-the-valley are rhizomes.



In a **corm**, food is stored in stem tissue. Corms look like bulbs, but if you cut a corm in half you see a mass of homogenous tissue rather than concentric rings of fleshy leaves as in a bulb. Cyclamen, gladiolus, taro (left), and crocus (right) are corms.



Some plants produce copies of themselves (tiny plantlets called bulbils) from axillary buds. In time, these fall off as independent plants. Examples include the hen and chicken fern (A*splenium bulbiferum*) (above) and kalanchoe (*Bryophyllum).*

Stolons (runners) are horizontal stems that grow above the ground. At certain points along the stolon, where it touches the ground, roots may form and a small plantlet appear. If the stolon breaks, the new plant becomes independent.

1. (a) What is meant by vegetative propagation? _____

_____

_____

(b) What feature of plant cells underlies this ability? _____

_____

ISBN: 978-1-98-856655-9

Taking advantage of vegetative structures for propagation

Many plants grown by humans are propagated from natural vegetative structures. Often, specific strains are grown from seed until vegetative structures are produced. These are then distributed to growers to produce the main crop.

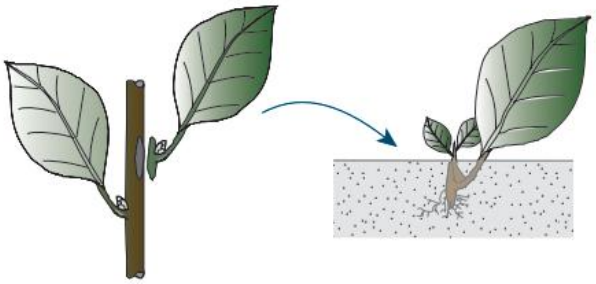
New potato hybrids are grown from seed. These produce tubers and the tubers are sold to farmers as seed potatoes. The seed potatoes produce a new plant and many more tubers, which are harvested for food.

Whole garlic bulbs are actually groups of individual garlic bulbs, called cloves. Each clove can be separated from the bulb, planted and grown to produce more whole garlic bulbs.

Sweet potatoes (*Ipomoea batatas*) are the root tubers of a vine that grows close to the ground (related to the morning glory). Like potatoes, the tubers are planted and produce more tubers that are then harvested.

Propagating plants from cuttings

Cuttings are sections of a parent plant, which are removed and grown as new individuals. These individuals will be clones (genetically identical copies) of the parent plant. Auxin is a plant hormone that accumulates at the base of the stem, triggering the formation of roots. Adding synthetic auxins to the end of the cutting promotes greater root development.



1. A leaf and accompanying axial bud are cut from the parent stock.
2. The cutting is placed in a growth medium containing rooting hormones and a new plant grows.

Melburnian

The spiny daisy (*Acanthocladium dockeri)* is a critically endangered daisy found in South Australia. It was first collected in 1860, in New South Wales, but by 1990 it was thought to be extinct. In 1999, five plants were found on a farm in South Australia. Cuttings were taken and the resulting plants have been successfully established around Banrock Station (SA).

2 (a) Describe some of the natural means of vegetative propagation in plants: _____

_____

_____

_____

(b) Can you think of one major advantage of having a reproductive structure filled with stored food (as in a tuber)?

_____

_____

3. Describe how plants benefit, generally, by reproducing vegetatively: _____

_____

_____

_____

4. Describe how humans have benefited from the vegetative propagation of plants: _____

_____

_____

_____

ISBN: 978-1-98-856655-9

7 Investigation into Plant Propagation

Key Idea: Different growing mediums may affect the ability of a plant to grow from cuttings.

Propagation is the process by which new plants are grown from a variety of sources, including seeds and cuttings. Most plants can grow from cuttings, although some plants are easier to propagate in this way than others. The growth medium can affect the cutting's ability to produce roots and start growing. Some cuttings will grow roots if the cut end is simply placed into a container of water. Cuttings from other plants have more specific requirements. In the experiment below, you will investigate the effect of different growing mediums on the production of roots from cuttings of the plant coleus (*Coleus scutellarioides*). This is a relatively common house plant that can be grown outside in warmer parts of Australia and produces roots from cuttings relatively easily.

Investigation 1.1 Plant propagation

See appendix for equipment list.


Sabina Bajracharya CC 4.0

1. You will need nine containers of equal size or a nine chambered planter, three planting mediums: washed sand, commercial potting mix, fine bark (or three mediums provided by your teacher), rooting hormone, and nine ice block sticks.
2. You will need to take nine cuttings from the coleus plant provided (or the plant provided by your teacher). Each cutting should have the same number of leaves (2-3) that are roughly the same size. Stems should be cut to the same length (about 10cm).
3. Fill three planting containers with one type of planting medium (e.g. sand), three more with the second type, and the last three containers with the third type.
4. For each container, add water so that the medium is slightly damp. Add the same amount of water to each of the nine containers and allow it to drain through if necessary.
5. Place a small amount of rooting hormone in a container and dip the cut end of each cutting into it, to the same depth.
6. Carefully push one cut stem into each container so that it can stand up on its own. For each container, use the ice block stick to label the container with your name/group and the planting medium.
7. Place the cuttings in a sunny position for several weeks, continuing to water the plants daily with the same amount of water in each container.
8. After 4-5 weeks, carefully remove one of the cuttings from a container. Be careful not to damage the roots. Carefully wash the planting medium off the cutting.
9. Count the number of roots emerging from the stem and record in the table below. Measure the length of each root and record in the table below.
10. Calculate the median number of roots and the median root length for each planting medium.

	Planting medium											
Pot	**1**	**2**	**3**	**Median**	**1**	**2**	**3**	**Median**	**1**	**2**	**3**	**Median**
Number of roots												
Length of roots (mm)												

1. (a) Is there a difference between the median number of roots and length of the roots between each planting medium?

(b) Explain your answer to 1(a):

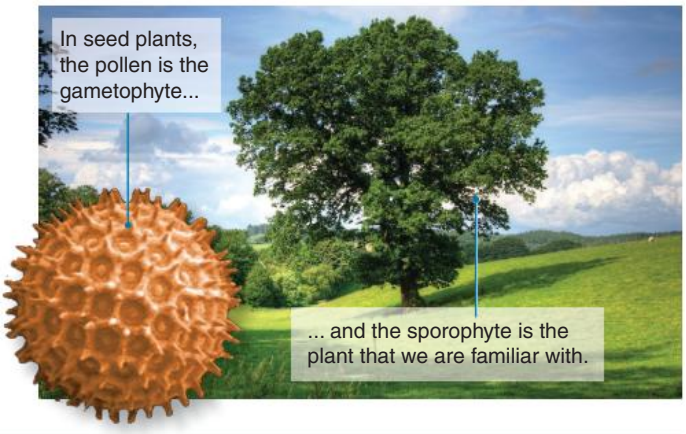
ISBN: 978-1-98-856655-9

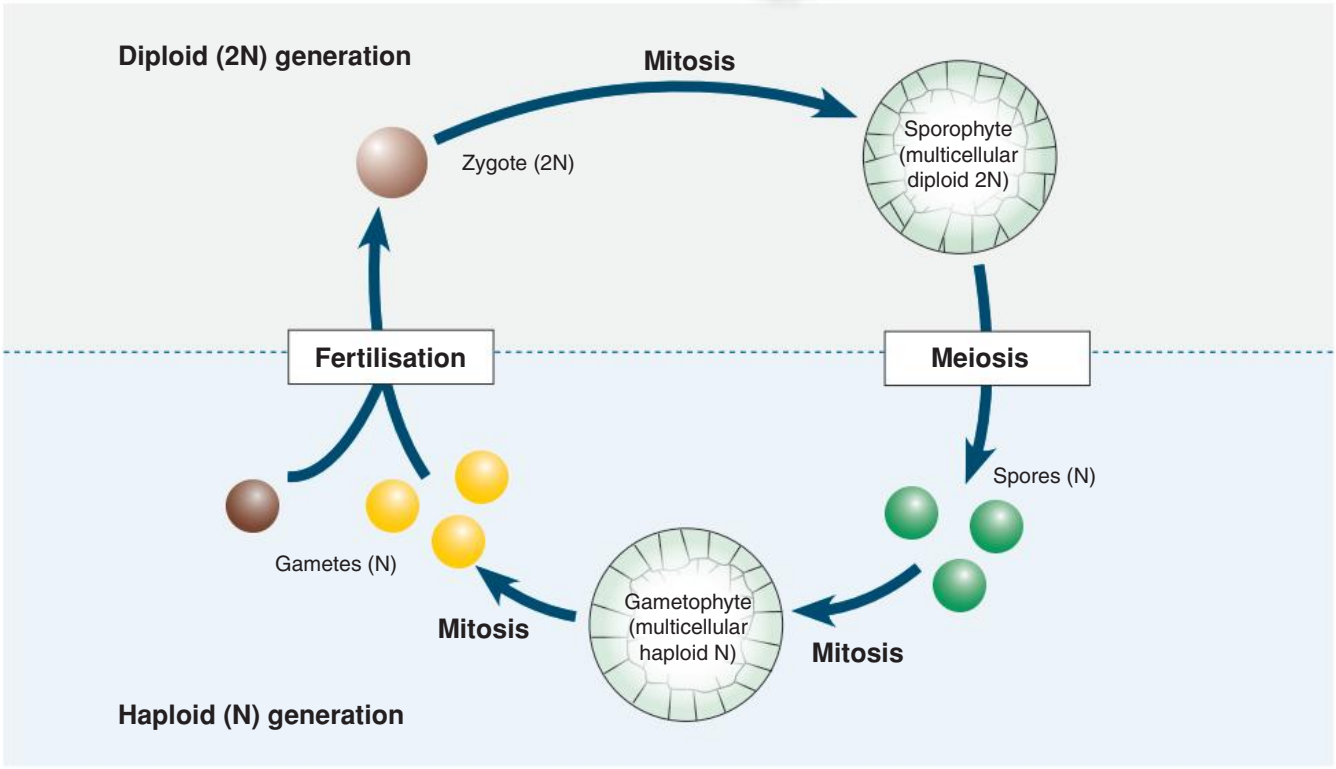
8 Features of Plant Sexual Reproduction

Key Idea: The life cycle of plants includes alternation between a haploid (N) gametophyte generation and a diploid (2N) sporophyte generation.

The life cycles of all plants are characterised by a phenomenon known as **alternation of generations.** The haploid (N) gametophyte generation alternates with the diploid (2N) sporophyte generation. In vascular plants, the sporophyte generation is dominant.

- The sporophyte and gametophyte generations are named for the type of reproductive cells they produce. Haploid gametophytes (one set of chromosomes, denoted N) produce gametes by mitosis, whereas diploid sporophytes (two sets of chromosomes, denoted 2N) produce spores by meiosis (production of cells for sexual reproduction).
- Spores develop directly into organisms. Gametes (egg and sperm) unite during **fertilisation** to form a **zygote,** which gives rise to an organism.
- Pollen carries the gametes from the male reproductive structures to the female reproductive structures.





1. Describe the alternation of generations in plants: _____

_____

_____

2. (a) Give an example of a sporophyte: _____

   (b) Give an example of a gametophyte: _____

3. Complete the table below to show what produces each structure:

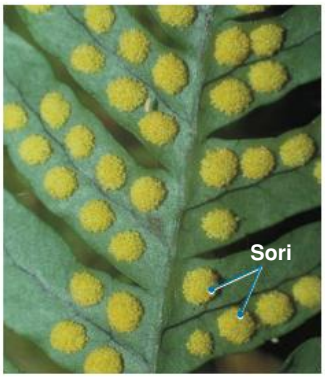
Structure	Spores	Gametes	Zygote
Produced by			
Process			

ISBN: 978-1-98-856655-9

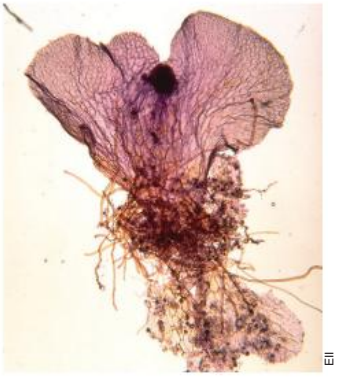
9 Reproduction in Ferns

Key Idea: In ferns, the gametophyte is a small, free-living plant that produces gametes that rely on water to unite. The sporophyte (the fern) produces haploid, unicellular spores. Reproduction in ferns is achieved by the alternating release of spores and gametes (there are no seeds). Spores from the sporophyte develop in sporangia, which are typically found on the underside of the fronds, in clusters called sori. Spores are single celled and small enough to be spread by the wind.

- If a fern spore settles in a suitable spot, it develops into a heart-shaped prothallus (the gametophyte), which is typically only one or two cells thick, small, free-living, and photosynthetic. It produces egg cells and motile sperm cells, which require a film of water to reach the egg cells.
- The division of the zygote begins immediately after fertilisation to produce the embryonic sporophyte. This becomes rooted in the soil and develops fronds. The gametophyte then disintegrates.

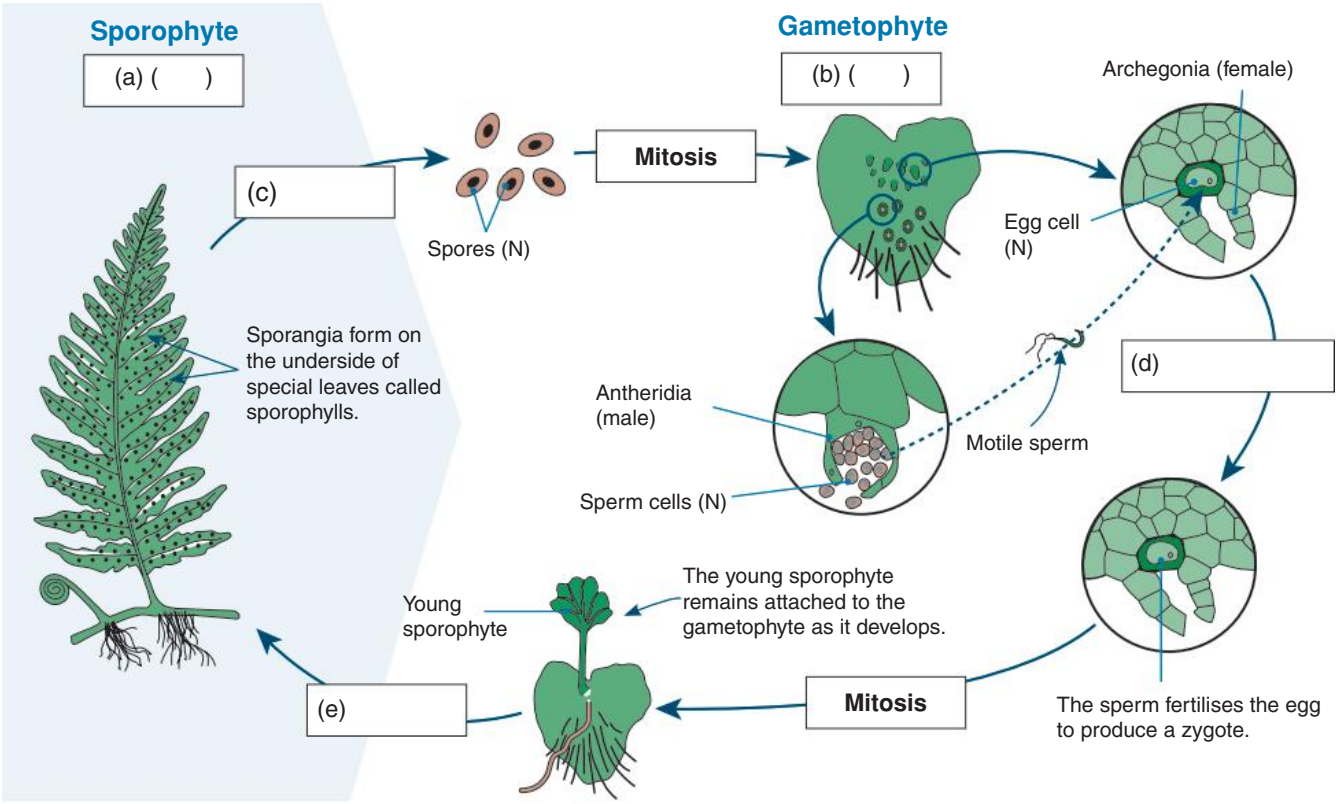


The spores are found in clusters underneath the frond, called sori.



The free living prothallus (the gametophyte) develops from the spore.

Life cycle of ferns



1. (a) - (e) Complete the diagram above, using the labels *N, 2N, fertilisation, meiosis, mitosis*:

2. What feature distinguishes ferns from seed plants (angiosperms and gymnosperms)? _____

_____

_____

3. (a) Why do ferns require moist environments in order to reproduce? _____

_____

(b) Explain why this requirement ultimately limits where they can survive in the long term: _____

_____

_____

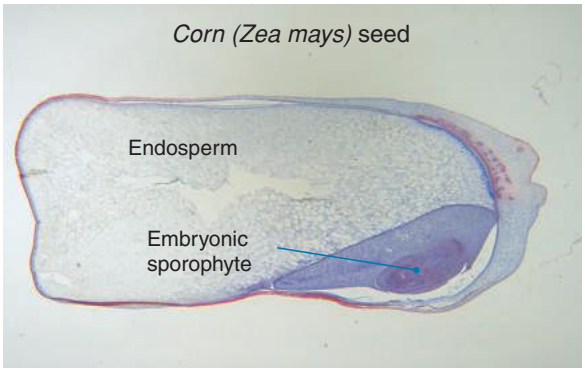
ISBN: 978-1-98-856655-9

10 Reproduction in Angiosperms

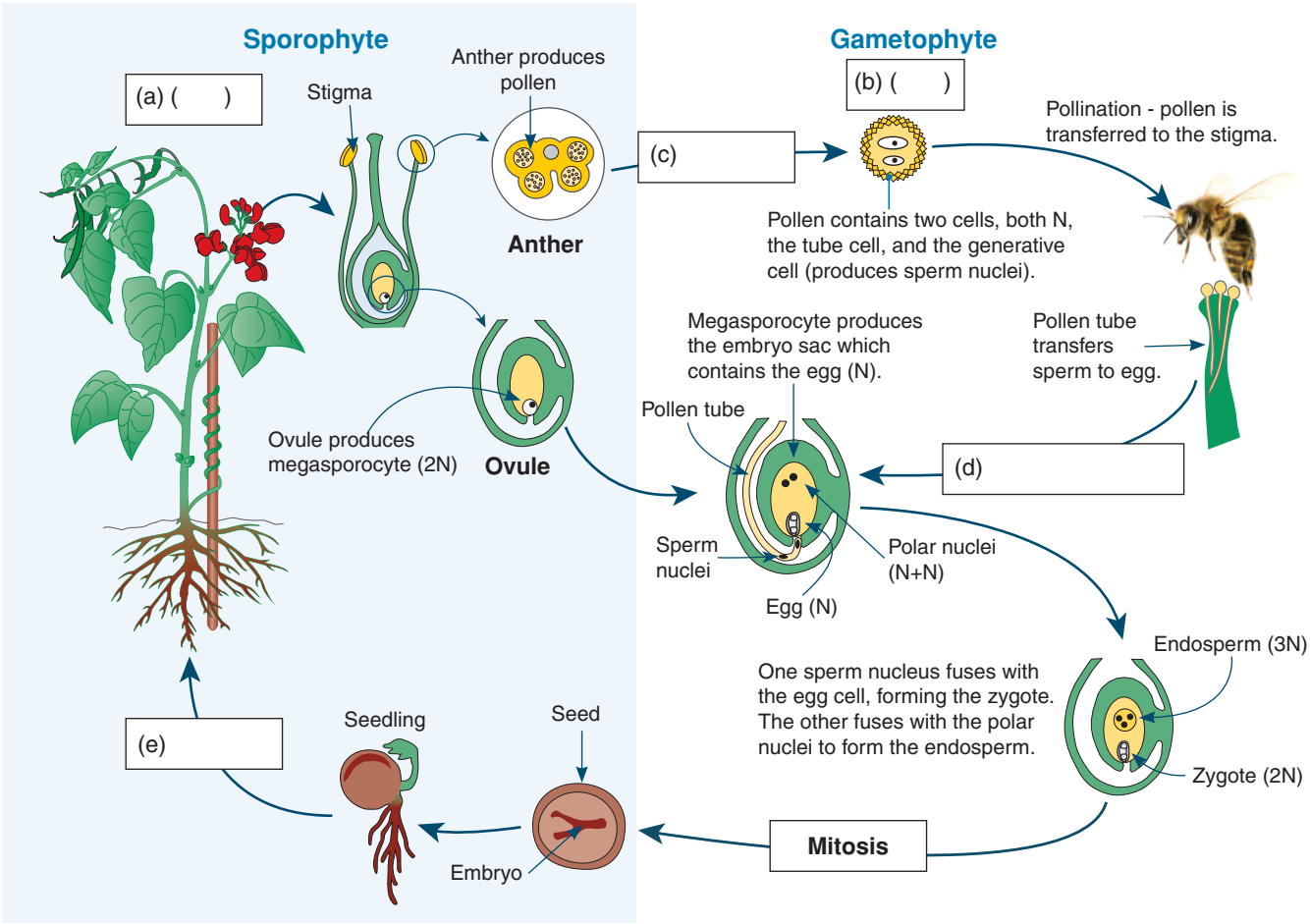
Key Idea: Angiosperms are flowering plants. The sporophyte produces flowers that contain the tiny gametophytes. Fertilisation of the gametes gives rise to the seed.

Angiosperms are flowering seed plants. In seed plants, pollen (not water) is the vehicle to carry the male gametes to the female gametes. The leafy plant that bears the flowers represents the sporophyte generation of the life cycle. The flowers contain specialised male and female structures (anthers and ovules), in which the male and female gametophytes are formed

- The pollen grain is the male gametophyte and the embryo sac is the female gametophyte. Each mature pollen grain contains two sperm nuclei and each mature embryo sac contains the egg and two polar nuclei.
- The typical life cycle of angiosperms (below) involves the formation of gametes (egg and sperm) from the haploid gametophytes, the fertilisation of the egg by a sperm cell to form the zygote, the production of fruit around the seed, and the germination of the seed and its growth by mitosis.
- The gametophyte generation may be important to a plant for reasons other than producing gametes. Endosperm (3N) derived from male and female gametophytes provides nutrients for embryonic sporophytes (right).



Life cycle of angiosperms (flowering plants)



1. (a) - (e) Complete the diagram above, using the labels *N, 2N, fertilisation, meiosis, mitosis*:

2. (a) In which part of a flowering plant do the male gametophytes develop? _____

   (b) In which part of a flowering plant do the female gametophytes develop? _____

3. Why don't angiosperms need a moist environment for fertilisation to occur? _____

_____

_____

ISBN: 978-1-98-856655-9

11 Reproduction in Gymnosperms

Key Idea: Gymnosperms are a group of seed plants that includes the conifers. The sporophytes produce cones, which produce either pollen or egg cells (not both).

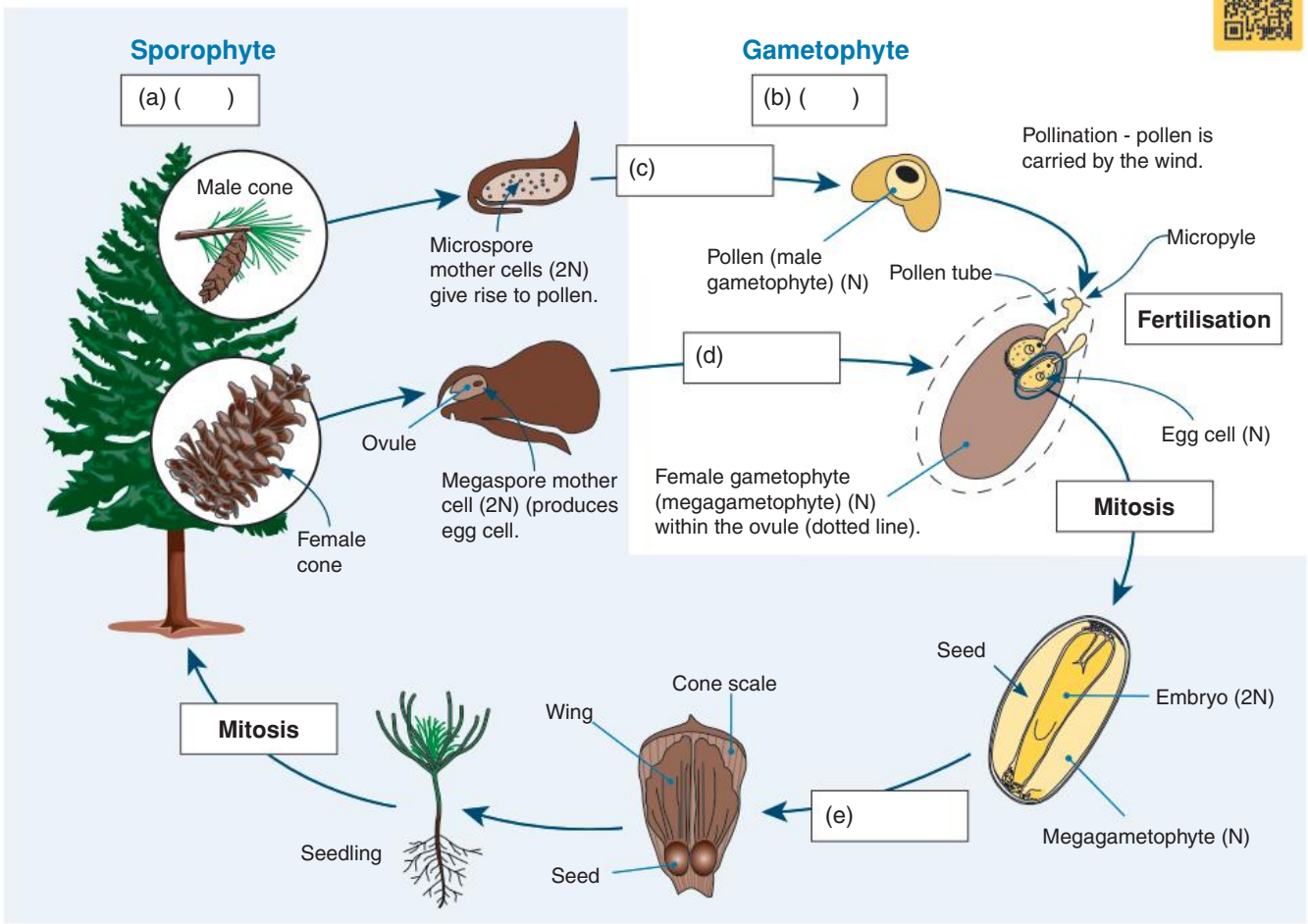
Conifers and cycads are gymnosperms. Conifers usually bear the smaller male and the much larger female cones on the same plant. Conifers are wind pollinated.

- All gymnosperms are wind pollinated. In conifers, the wind-carried pollen sticks to a drop of fluid within a tiny hole (the micropyle) at the end of the ovule. When the fluid evaporates, it draws the pollen into the ovule. The pollen develops a pollen tube, which grows towards the egg cell (this can take up to 15 months).
- After fertilisation, a zygote forms which develops into an embryo then into a seed, which is often 'winged'. Some conifers require the intense heat from a fire to open the cones and release the seeds.


Male cones


Female cones

Life cycle of conifers (cone-bearing plants)



1. (a) - (e) Complete the diagram above, using the labels *N, 2N, meiosis, mitosis*:

2. Briefly describe how the sperm cell reaches the egg cell in conifers: _____

_____

_____

3. Which structure in a conifer is equivalent to the embryo sac of angiosperms? _____

4. What is the role of wind in the complete life cycle of a conifer? _____

_____

ISBN: 978-1-98-856655-9

12 Insect Pollination

Key Idea: Many angiosperms have a mutualistic relationship with their pollinators in which the plants achieve pollination by rewarding insects with food such as nectar or pollen.

Pollination is the transfer of pollen from the male anther to the female stigma. Pollination of flowers by insects is usually mutualistic. Mutualistic relationships involve exchanges between two species, so that each species benefits. The benefit need not be equal for each party because each species acts in its own interests. In the case of insect pollination, the insect benefits from the energy in the plant nectar or pollen it consumes. The plant benefits by having its gametes transferred to another plant. Nearly 88% of all flowering plants are pollinated by animals, with the vast majority being insects.

Cross section of an insect pollinated flower

Female Reproductive Structures

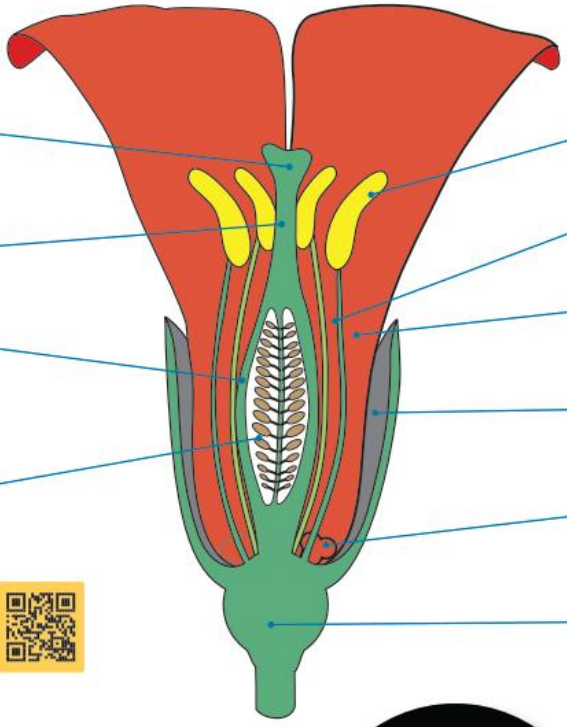
Stigma: The receptive part of the carpel. Pollen grains will germinate only if they land here.

Style: The structure that supports the stigma.

Ovary: The base of the carpel where the ovules develop.

Ovules: These are eggs and once fertilised, become the seeds. The ovule skin becomes the seed coat or testa.

An entire female part is the carpel. There may be one or more carpels per flower.



Male Reproductive Structures

Anther: Top portion of the stamen, the male organ of reproduction.

Filament: The slender stalk of the stamen that supports the anther.

Petals: Collectively, these form the corolla. Often brightly coloured.

Sepals: Together form the calyx. Usually green, but sometimes the same colour as the petals.

Nectary: Plants produce a sugary liquid called nectar to attract insects to the flower.

Receptacle: The swollen base of the flower. Sometimes it forms the succulent tissue of the fruit.

The petals of flowers guide insects towards the pollen or nectar at the centre of the flower, using various colours and lines known as nectar guides. In this way, wandering insects are enticed into entering the flower and transfer pollen in the most efficient way.

Bees, and many other insects, are able to detect ultraviolet light. Many flowers contain pigments that reflect UV, producing a specific pattern visible to insects but not to other animals. In this way, plants can use their flowers to specifically attract preferred insect pollinators.

1. Describe the difference between the stigma and the anther: _____

_____

2. (a) Explain why flowering plants need to attract pollinators to their flowers: _____

_____

_____

(b) Describe the adaptations in angiosperms that attract specific pollinators to their flowers: _____

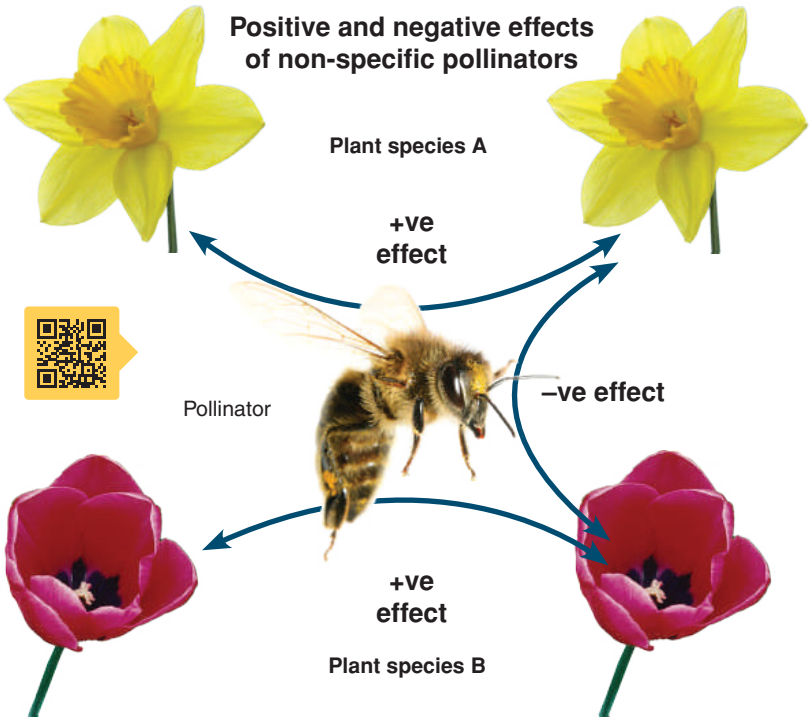
_____

_____

_____

_____

ISBN: 978-1-98-856655-9



Most insect pollinators are generalists, meaning they do not form pollination relationships with specific plants. Honey bees, for example, pollinate many different kinds of plants. This can be of negative value to a particular plant, as the energy expended in producing pollen and nectar is wasted if the bee does not then fly to a plant of the same species.

A mutualism is **facultative** when the species involved do not rely on either for survival, but take advantage of each other, when present. Many insects will take pollen and nectar from many plant species and do not rely on just one for survival.

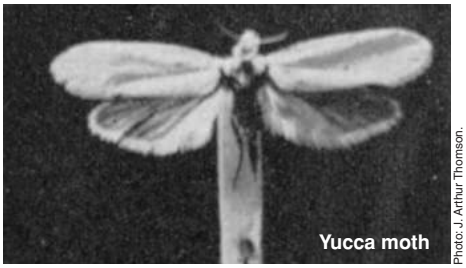


Photo: J. Arthur Thomson.

Obligate mutualism occurs when one species relies entirely on another for its survival. One example is the relationship between the yucca and the yucca moth. The yucca cannot make seeds without the moth, and the moth larvae only eat yucca seeds.

Orchid flowers are highly variable in their structure, and often highly specialised. They often have only one specific insect pollinator. This relationship is the result of the plant and its pollinator evolving together.

Magnolias are an ancient plant group with generalised flowers. They evolved before bees and evolved with beetles as their main pollinators. Magnolias produce large amounts of pollen, some of which is food for the beetles.

Angiosperms may not always have been the only plants to rely on insect pollination. Gymnosperms may have been pollinated by scorpionflies (above) in the Jurassic, long before flowers evolved.

3. Describe the benefits of **mutualism** to both the flower and the pollinator: _____

4. Discuss the advantages and disadvantages of flowers having obligate pollinators: _____

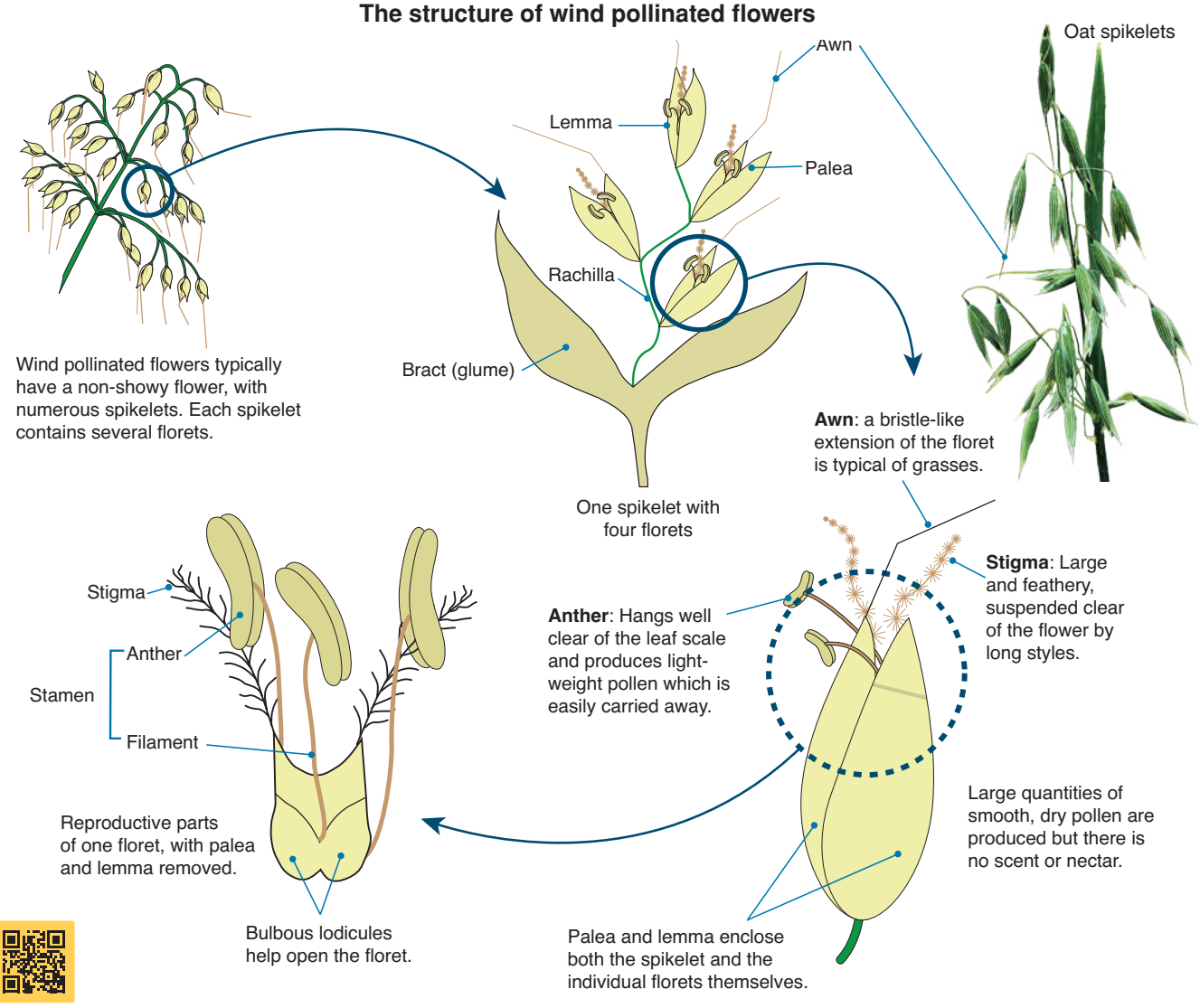
ISBN: 978-1-98-856655-9

13 Wind Pollination

Key Idea: The flowers of wind pollinated plants are usually small and pale coloured. Vast quantities of pollen are needed to achieve successful cross-pollination.

The flowers of wind pollinated plants are not as large or brightly coloured as those of insect pollinated plants. Their flowers are generally small and may lack petals altogether, while the anthers and stigma are large and hang clear of the surrounding structures. Wind pollen is highly inefficient, with most pollen falling to the ground within 100m, so large amounts of pollen are produced to ensure successful cross pollination with other plants. All gymnosperms, but only around 10% of flowering plants, are wind pollinated. However, this 10% includes the grasses, which are one of the most successful of all the angiosperm groups.

The structure of wind pollinated flowers



As with most wind-pollinated plants, grass flowers are small, but produced in large numbers. The arrow in the photograph above points to the anthers of a grass plant on the flowers, which are held above the ground on a stalk.

The flowers of wind-pollinated trees are small, often with green petals, and very numerous. Wind pollinated trees are common in temperate regions where wind movement through deciduous trees easily disperses the pollen.

Gymnosperms are all wind pollinated. In conifers, the male cones are often borne on the lower branches. They produce vast quantities of pollen, which must be blown upwards towards female cones in higher branches of other trees.

ISBN: 978-1-98-856655-9

1. Describe three differences between the insect and wind pollinated flowers shown in the photographs below:

   (a) _____

   (b) _____

   (c) _____


Insect pollinated plant


Wind pollinated plant

2. Using the photographs and your answers above to help you, contrast wind and insect pollinated flowers with respect to each of the following characteristics. For each, give reasons for the differences observed:

   (a) Appearance of the flowers: _____

   (b) Production of scent and nectar: _____

   (c) Amount of pollen produced: _____

   (d) Position of the reproductive parts (stigma, stamens): _____

3. Describe two adaptations of wind pollinated flowers:

   (a) _____

   (b) _____

4. Contrast the efficiency of wind and animals as pollinating agents, giving a reason for your answer: _____

5. Describe the main advantage of cross-pollination: _____

ISBN: 978-1-98-856655-9

14 Pollination and Fertilisation

Key Idea: In plants, pollination is essential to ensure fertilisation and production of seeds.

Pollination is the transfer of pollen grains from the male reproductive structures to the female reproductive structures of plants. This must happen before **fertilisation** (the joining of the egg and sperm) can occur. Adaptations to ensure **cross-pollination** (pollination between different plants) include structural and physiological mechanisms associated with the flowers or cones themselves, and reliance on wind and animal pollinators.

Mechanisms to ensure cross pollination

Male willow catkin

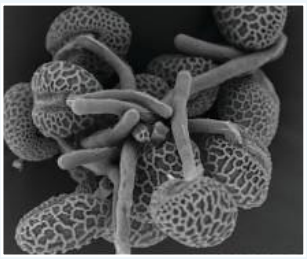
An effective way of ensuring cross-pollination is to have separate male and female plants. This occurs in about 6% of angiosperms including willows and holly.

Male and female pine cones

Other plants produce separate male and female flowers or cones on the same plant. They may develop at different times to ensure that pollen does not fertilise the same plant.

Tulip anthers and stigma

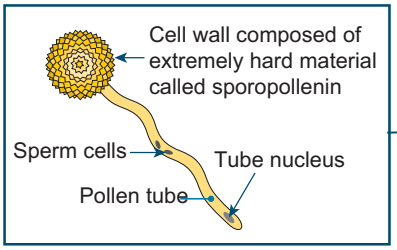
Some angiosperms have flowers with both male and female structures. They can ensure cross-pollination if the anthers and stigma mature at different times.

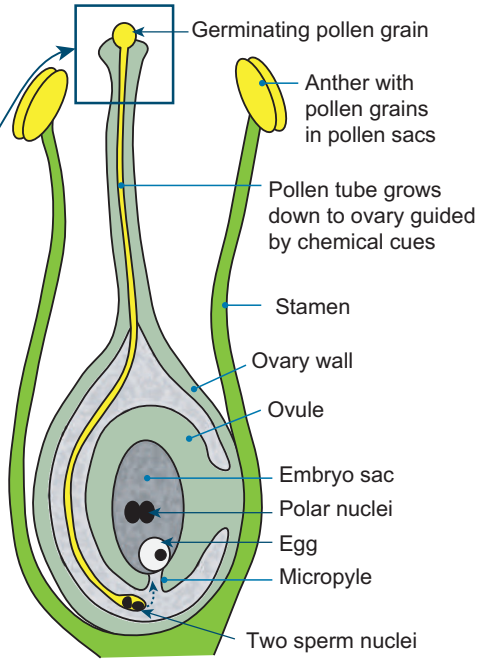


Germinating pollen grains

In many plants, pollen landing on the stigma of the same plant will not even germinate. This ensures that the egg cells are not fertilised by sperm from the same plant.

- Pollen grains are immature male gametophytes, formed by mitosis of haploid microspores within the pollen sac. Pollination is the actual transfer of the pollen from the stamens to the stigma, or from the male cone to the female cone. Pollen grains cannot move independently. They are usually carried by wind or animals.





Angiosperm

Fertilisation in angiosperms

- In **angiosperms,** pollen lands on the sticky stigma and completes development, germinating and growing a pollen tube that extends down to the ovary (shown right). The pollen tube is directed by chemicals (usually calcium) to the ovule. It enters through the micropyle, a small gap in the ovule. A **double fertilisation** takes place. One sperm nucleus fuses with the egg to form the zygote. A second sperm nucleus fuses with the two polar nuclei within the embryo sac to produce the endosperm tissue (3N). There are usually many ovules in an ovary, therefore many pollen grains (and fertilisations) are needed before the entire ovary can develop.

1. Explain why plants have generally evolved to limit self-pollination: _____

_____

2. Describe three ways in which plants avoid self-pollination:

(a) _____

_____

(b) _____

_____

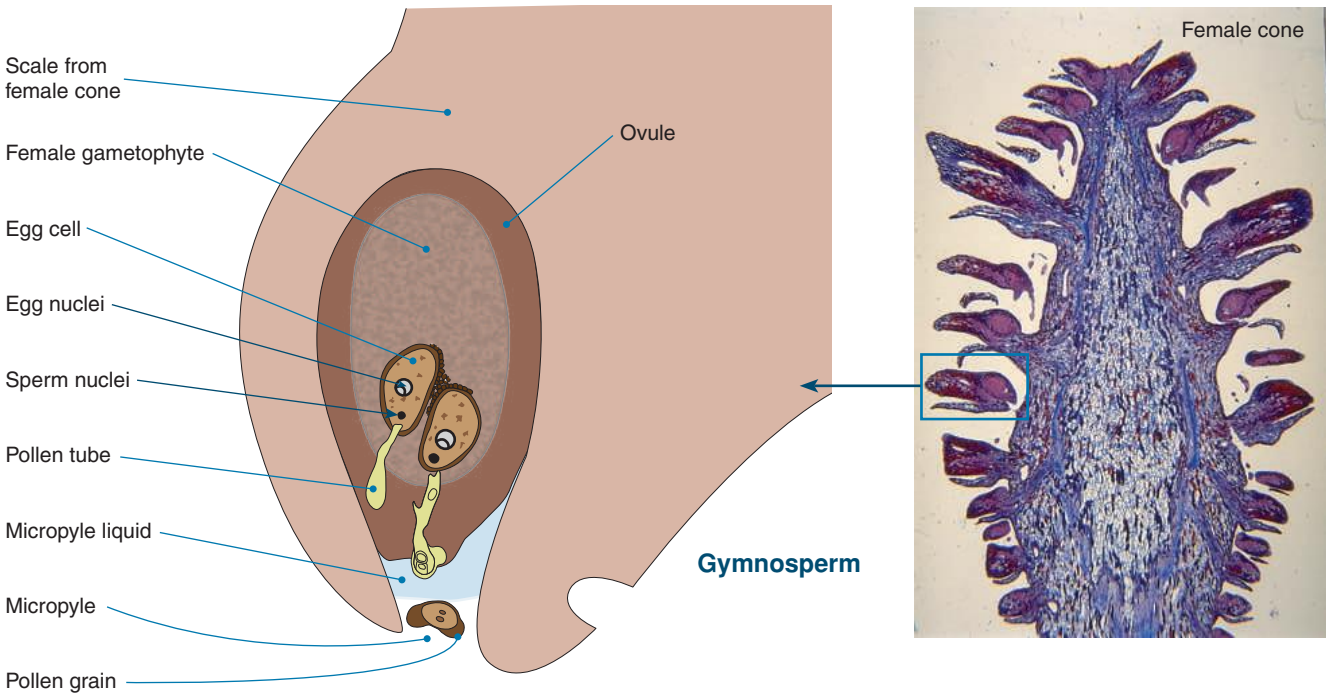
(c) _____

_____

ISBN: 978-1-98-856655-9

Fertilisation in gymnosperms

- In conifers, the pollen lands on a drop of fluid covering the micropyle and is drawn down into the micropyle to the ovule as the fluid evaporates. The pollen tube germinates shortly afterwards, but may take 15 months to reach the egg cell. The pollen grain produces the sperm-producing cell which produces two sperm nuclei. One nucleus fuses with the egg cell while the other degenerates. About 4% of seeds contain multiple embryos, which are formed when more than one egg cell in the ovule develops.



3. Describe the mechanisms to ensure cross-pollination in plants: _____

4. Distinguish clearly between pollination and fertilisation: _____

5. Briefly describe how pollination differs between angiosperms and gymnosperms: _____

6. Describe the role of the double fertilisation in angiosperms and explain how this is different to gymnosperms:

7. Name the main chemical responsible for pollen tube growth: _____

8. Explain what would happen if a seed containing multiple embryos germinated: _____

ISBN: 978-1-98-856655-9

15 Seed Structure and Germination

Key Idea: The seed houses the dormant embryonic plant and its food store until conditions for germination are met.

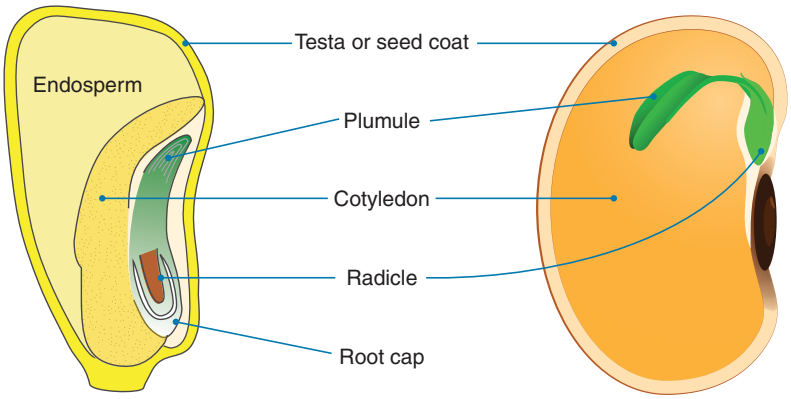
After fertilisation, the ovules within the ovary become the **seeds**. Recall the double fertilisation in angiosperms; one fertilisation produces the embryo and the other produces the endosperm. The development of the endosperm is important as it provides a nutrient store for the young plant. A seed is an entire reproductive unit, housing the embryonic plant in a state of dormancy. During the last stages of maturing, the seed dehydrates until its water content is only 5-15% of its weight. The embryo stops growing and remains dormant until the seed germinates. At germination, the seed takes up water and the food store is mobilised to provide the nutrients for plant growth and development.

USDA

Dicot seeds: soy (above) **cashew** (below) There are two fleshy cotyledons. These store food absorbed from the endosperm.

In **germination**, the seed must rehydrate and reactivate its metabolism. The seed absorbs water through the seed coat (testa) and micropyle. As the seed tissue takes up water, the cells expand, metabolism is reactivated, and growth begins. Activation begins with the release of the hormone gibberellin (GA) from the embryo. GA promotes cell elongation, so the root can penetrate the testa. It also stimulates the synthesis of enzymes which hydrolyse the starch to produce sugars. The mobilised food is delivered to the developing roots and shoots.

Seed structure and function



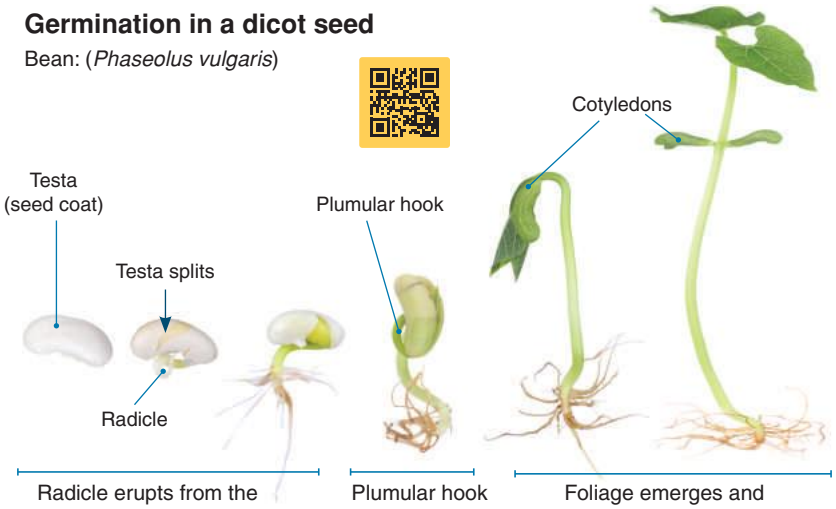
Monocot seed
Maize: (*Zea mays*)

Dicot seed
Bean: (*Phaseolus vulgaris*)

Every seed contains an embryo comprising a rudimentary shoot (plumule), root (radicle), and one or two cotyledons (seed leaves). The embryo and its food supply are encased in a tough, protective seed coat or **testa**. In monocots, the endosperm provides the food supply, whereas in most dicot seeds, the nutrients from the endosperm are transferred to the large, fleshy cotyledons.

Germination in a dicot seed

Bean: (*Phaseolus vulgaris*)



Radicle erupts from the seed and grows rapidly downwards.

Plumular hook protects the emerging stem.

Foliage emerges and secondary roots emerge from the lateral roots.

1. What is the purpose of a seed? _____

_____

2. (a) State the function of the endosperm in angiosperms: _____

   (b) State how the endosperm is derived: _____

3. What is the role of the testa? _____

4. Explain why the seed requires a food store: _____

_____

5. Why must stored seeds be kept dry? _____

ISBN: 978-1-98-856655-9

Investigating the effect of water on germination

- There are many factors affecting the germination of a seed. How those factors affect germination varies from species to species. In general there are three requirements for seed germination: water (absorption and reactivation of metabolism), oxygen (for cell respiration), and a temperature that allows metabolism to proceed. Light may or may not be required for germination, depending of the species, although light is required very soon after germination.
- Water is essential for the germination process. It enables expansion of the growing cells and activates the enzymes needed for germination. It is also needed for the hydrolysis of stored starch and the mobilisation of food molecules.

Investigation 1.2 Germination investigation

See appendix for equipment list.

1. You will need four planting containers and four sets of tomato seeds, (or other small, relatively quickly germinating seeds e.g. mustard seeds), and suitable sterilised growing medium. There should be about 20 seeds in each set (numbers may vary, as the result can be pooled as a class, but around 100 seeds in total per set should be used).
2. Put one set of seeds aside. Soak the other three sets of seeds in water at room temperature for 12 hours, 24 hours, or 36 hours (or other equally spaced time, based on lab availability). Begin the soaking process so that all three sets of seeds finish their soaking at the same time.
3. Plant each of the four sets of seeds in one of the four containers, spacing the seeds in a grid at equal distances from each other. Place the seeds in their containers, in a sunny position, at room temperature.
4. For 12 days, water each set of seeds with 500ml of water per day, allowing to the water to drain thoroughly.
5. Count the number of seeds germinated each day, and record.

	Days after soaking												
Soaking duration	1	2	3	4	5	6	7	8	9	10	11	12	Total germinated
0													

6. Plot the result on the grid above:
7. At which length of soaking time did the greatest number of seeds germinate? _____
8. Identify one way in which the experiment could be made more accurate: _____

_____

9. Identify one other experiment that could be done to extend this initial experiment: _____

_____

ISBN: 978-1-98-856655-9

16 Seed Dispersal

Key Idea: Seeds are dispersed from the parent plant to reduce competition for light and nutrients.

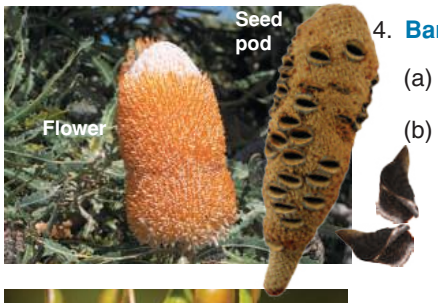
Plants disperse their seeds to expand their range and reduce competition. In some cases, the seed itself is the agent of dispersal, but often it is the fruit or an associated attached structure. Seeds are mainly dispersed by wind, water, and animals. Wind dispersed seeds have wing-like or feathery structures that catch the air currents and carry the seeds. Plants that rely on animals to spread their seeds may have hooks or barbs that catch the animal hair, sticky secretions that adhere to the skin or hair, or fleshy fruits that are eaten, leaving the seed to be deposited in faeces, away from the parent plant. Other dispersal mechanisms rely on explosive discharge or shaking from pods or capsules.

For each of the examples below, describe the method of dispersal and the adaptive features associated with the method:

1. **Dandelion** seeds are held in a puff-like cluster:
   (a) Dispersal mechanism: _____
   (b) Adaptive features: _____

2. **Acorns** are heavy fruits in which the fleshy seeds are encased in a resistant husk:
   (a) Dispersal mechanism: _____
   (b) Adaptive features: _____

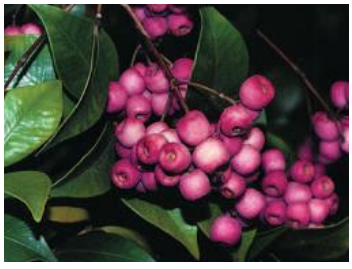
3. **Coconuts** are heavy buoyant fruits with a thick husk:
   (a) Dispersal mechanism: _____
   (b) Adaptive features: _____



Changarra CC 2.5

4. **Banksia** seeds are attached to a light wing structure:
   (a) Dispersal mechanism: _____
   (b) Adaptive features: _____

5. **Wattle** (*Acacia* spp.) seeds are enclosed in pods. A fleshy strip surrounds each seed:
   (a) Dispersal mechanism: _____
   (b) Adaptive features: _____



6. **LillyPilly** (*Acmena* spp., *Syzgium* spp.) seeds are surrounded by fleshy fruits:
   (a) Dispersal mechanism: _____
   (b) Adaptive features: _____

ISBN: 978-1-98-856655-9

17 Animal Sexual Reproduction

Key Idea: Fertilisation refers to the union of male and female gametes to form a zygote. In animals, both fertilisation and development may occur externally or internally.

Sexual reproduction involves the production of sex cells (gametes) produced by sex organs (gonads). Female gametes are called eggs, male gametes are called sperm. Fertilisation in animals can be either internal or external. Many aquatic invertebrates and fish use external fertilisation, where the parents release their gametes into the water at the same time. Other invertebrates, reptiles, sharks, birds, and mammals use internal fertilisation, where sperm is transferred directly into the female to increase the chances of successful fertilisation. In birds and most reptiles, one adaptation to life on land has been the evolution of the amniote egg. The egg enables the embryo to complete its development outside the parent's body, inside a protective shell, and nourished by a yolk sac. The pattern of internal development in mammals, termed gestation or pregnancy, provides the most advantages for the embryo, in terms of nourishment and protection during development.

External fertilisation

External fertilisation

External fertilisation has many disadvantages. Sperm may not efficiently reach eggs or environmental conditions may not favour fertilisation. Amphibious creatures, e.g. frogs, must return to water in order to reproduce, limiting their ability to live away from water. Many marine creatures must produce huge volumes or numbers of eggs and sperm, e.g. giant clam (above left), and this uses valuable resources. However, external fertilisation is behaviourally simpler than internal fertilisation and, in some cases, does not require the physical meeting of male and female.

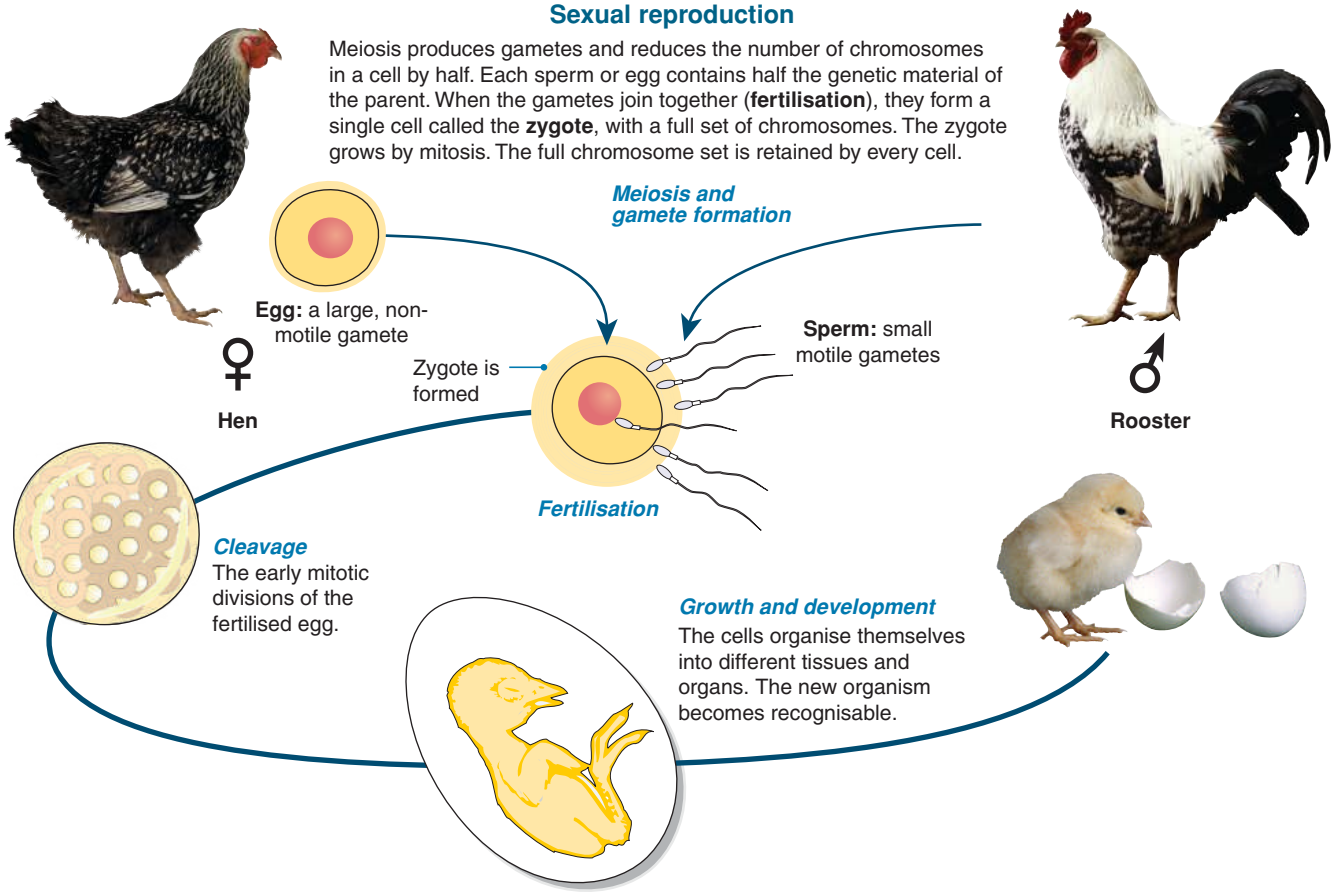
Internal fertilisation

Internal fertilisation

Internal fertilisation is more efficient than external fertilisation. The effect of the environment on the gametes is removed and the developing offspring, or eggs, are protected until mature. Fewer eggs and sperm need to be produced. However, there are disadvantages. Individuals must come into close contact. Elaborate courtships are often needed to communicate intentions and prevent attack by one individual on the other. A limited number of offspring or eggs may be produced, compared to external fertilisation.

Sexual reproduction

Meiosis produces gametes and reduces the number of chromosomes in a cell by half. Each sperm or egg contains half the genetic material of the parent. When the gametes join together (**fertilisation**), they form a single cell called the **zygote**, with a full set of chromosomes. The zygote grows by mitosis. The full chromosome set is retained by every cell.

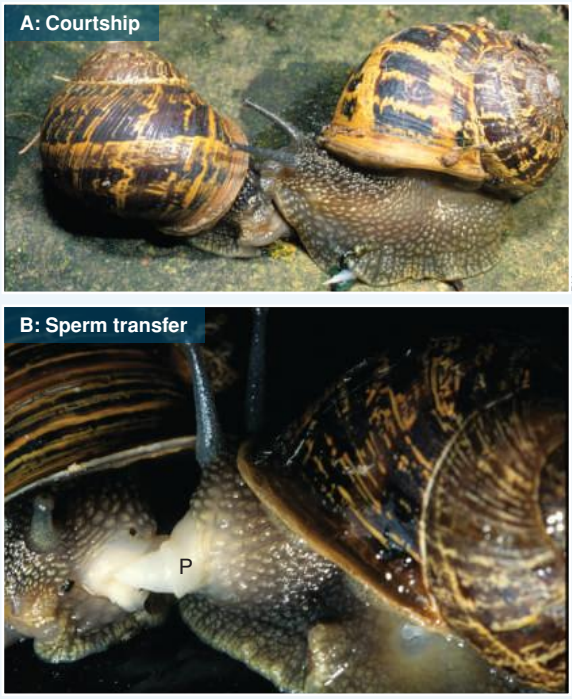


ISBN: 978-1-98-856655-9

Hermaphroditism

Most animals have separate sexes, i.e. individuals are either male or female. However, in some animals both sperm and eggs can be produced in the same individual. Such animals are known as hermaphrodites. In earthworms (below), flatworms, and some molluscs, e.g. land snails, both male and female organs are active in the same animal, and there is typically a reciprocal transfer of sperm, i.e. each receives sperm from the other during copulation. In this type of hermaphroditism, there is no self-fertilisation; a mate is necessary for any fertilisation to occur. However, some specialised hermaphroditic animals, such as parasitic tapeworms, are capable of self-fertilisation.

The photo above shows two earthworms in a mating clasp. Each worm places its reproductive region, the clitellum, against the reproductive region of the other worm, and sperm are exchanged.



Courtship and mating in the snail *Cantareus aspersus* (formerly *Helix aspersa*, (above)). During an elaborate courtship (A), calcareous darts are fired from the genital opening (behind the tentacle) into the body of the partner. Mating (B) involves reciprocal transfer of sperm via a penis (P).

1. Describe one advantage of sexual reproduction: _____

2. Describe one potential disadvantage of sexual reproduction: _____

3. For the bird example on the previous page, compare the key differences between male and female gametes:

   (a) The size of gametes: _____

   (b) Number of gametes produced: _____

   (c) Motility of gametes: _____

4. Distinguish between internal fertilisation and external fertilisation, identifying advantages of each strategy:

5. Describe two features of the amniote egg, e.g. in birds, that make it an ideal adaptation to reproduction on land:

   (a) _____

   (b) _____

ISBN: 978-1-98-856655-9

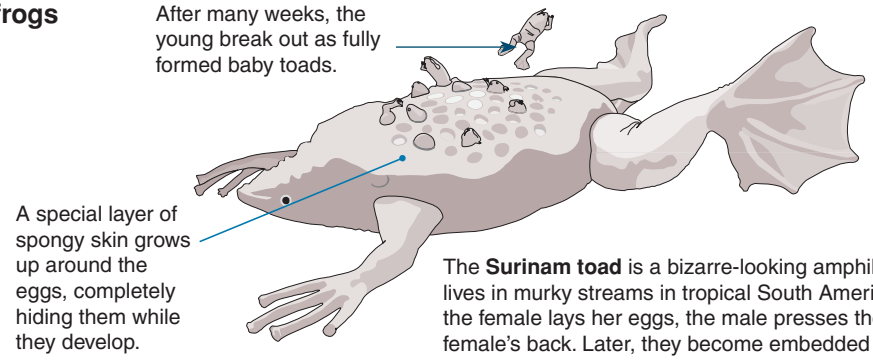
18 Animal Reproductive Strategies

Key Idea: Animals have evolved diverse structural, physiological, and behavioural strategies to maximise the production of viable young.

To reproduce sexually, animals must have systems to ensure that gametes meet, fertilisation takes place, and the zygote develops successfully. Invertebrates have evolved some of the most diverse reproductive systems. Most insect species lay eggs, with a very few giving birth to live young. Amongst the vertebrates, the basic structures of the reproductive system are relatively uniform, but there is huge variation in the strategies shown by different vertebrate groups. Biologists distinguish oviparous (egg laying) animals from viviparous (live bearing) animals. A small number of vertebrates, including some snakes, are ovoviviparous, i.e. the young are born live, but from eggs hatched within the parent's body. Reproductive strategies also include the effort involved in caring for offspring. There may be many offspring produced but little parental care, or few offspring with much parental care.

Reproductive strategies of frogs

- Frogs provide a good example of the variety of strategies that are employed to ensure successful reproduction. There are about 40 known reproductive methods used by frogs and toads, including simply laying eggs in water to develop on their own, carrying the eggs or tadpoles of the frog's back, and gastric brooding.



The **Surinam toad** is a bizarre-looking amphibian that lives in murky streams in tropical South America. When the female lays her eggs, the male presses them into the female's back. Later, they become embedded in the back as the skin swells up around the eggs.

Some frogs lay their eggs in a nest of foam either on land (attached to leaves) or floating on water. The foam not only hides the eggs from predators, but it keeps them moist and prevents them from drying out.

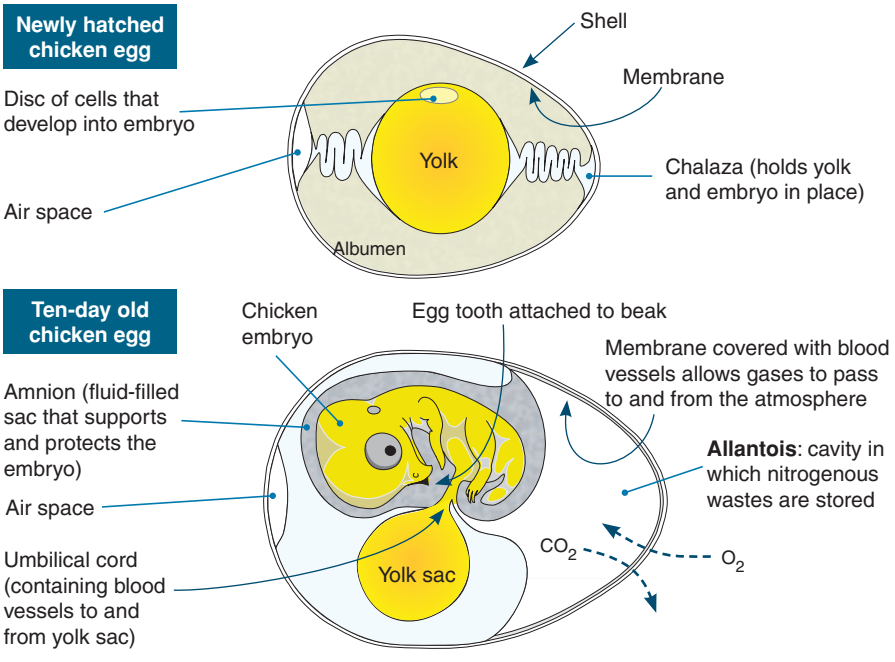
Some species of small frogs in both South America and Africa lay eggs that hatch into tadpoles on land. The tadpoles stick to the back of one of the parents with mouthparts modified to function as suckers. The parent carries the tadpoles to water.

Some frog species lay eggs on leaves or branches overhanging the water. In some of these species, one of the parents remains with the eggs until they hatch. The tadpoles that emerge from the eggs drop into the water below to complete their development.

When a female midwife toad lays her string of eggs, the male winds them around his back legs. He carries the eggs for about a month, visiting puddles to keep them moist. When the eggs are ready to hatch he places them in a suitable pool.

The structure and function of eggs

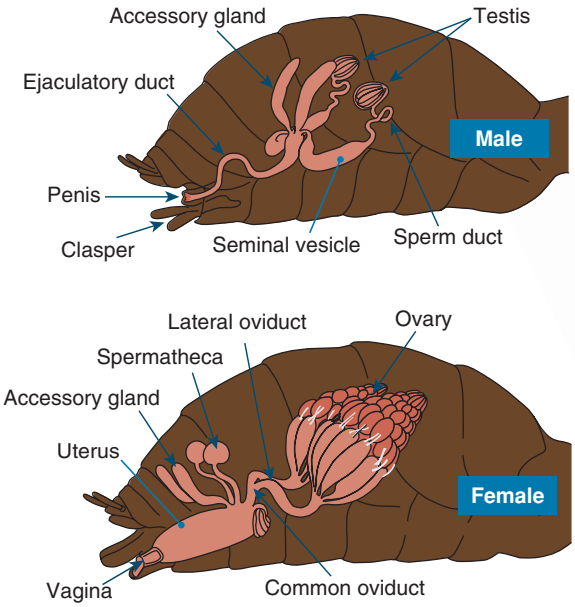
- Both reptiles and birds (which evolved from reptilian ancestors) develop watertight shelled eggs. The egg is supplied with all the necessary food material as well as fats that yield water when metabolised.
- The shell enclosing the egg provides protection and reduces water loss, yet permits gas exchange. Waste materials from the developing chick embryo are stored in the egg.
- In birds, laying eggs allows the parent to continue to fly without the effort of carrying extra weight.
- Reptile eggs have a soft, somewhat rubbery shell, while birds' eggs have a hard, calcium carbonate shell.



ISBN: 978-1-98-856655-9

Reproductive strategies of insects

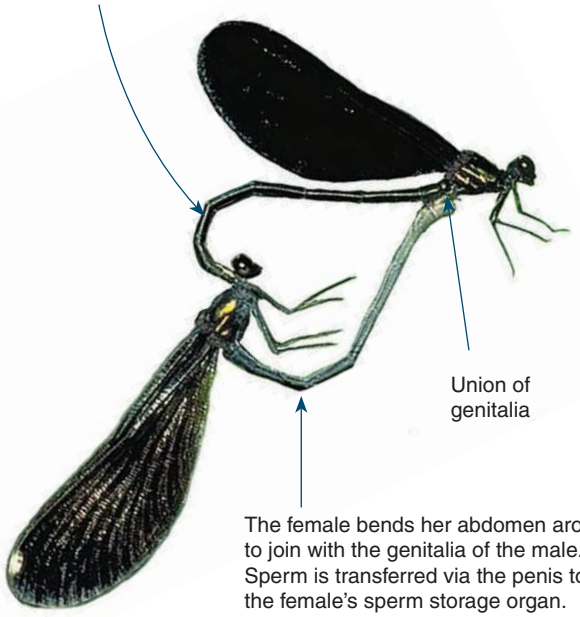
- Insects reproduce sexually, mainly by internal fertilisation followed by the production of yolk-filled eggs. A single pair of gonads is located in the abdomen. Most insects transfer sperm within small packets called spermatophores. Claspers at the end of the male's abdomen hold the female's abdomen during copulation. The terminal segments of the female's abdomen may form an ovipositor, which is often extendable or needle-like, with which they lay their eggs. Depending on the insect species, eggs may be buried in soil, animal dung, or rotting carcasses, injected into plant tissue or living hosts, or cemented to twigs or leaves.



Mating between damselflies

- The Odonata (damselflies and dragonflies) are unique amongst the insects. The male's penis is situated on the abdomen, close to the thorax. However, the testes are located at the end of the abdomen so that, before mating, the male has to bend its abdomen around to transfer semen from the testes to the penis. The location of the male genitals also accounts for the unique 'wheel position' adopted by Odonata, as shown below.

The male damselfly grips onto a twig with its front legs during mating while holding the female behind the head with clasping organs at the end of its abdomen.



1. Giving examples, distinguish between oviparous and viviparous vertebrates: _____

_____

_____

2. Some frogs and toads have evolved novel ways of enhancing the survival of their eggs.

   (a) How does the midwife toad enhance the survival of its eggs? _____

   _____

   (b) How does the Surinam toad enhance the survival of its eggs? _____

   _____

3. (a) Name two vertebrates that produce shelled, waterproof eggs: _____

   (b) Which feature of a vertebrate egg has been primarily responsible for its success? _____

   _____

   (c) Explain how a vertebrate egg provides for the following needs of a developing embryo:

   Elimination of wastes: _____

   _____

   Gas exchange: _____

   _____

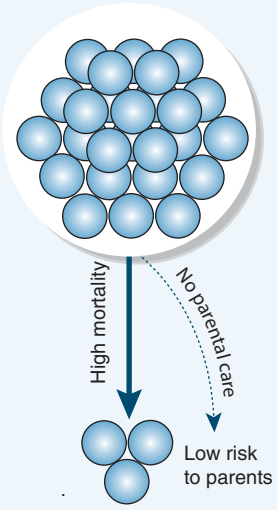
   Nutrition (food supply): _____

ISBN: 978-1-98-856655-9

Reproductive strategies

- The reproductive effort is the amount of energy allocated to reproduction, i.e. the production and care of young. The way in which an animal allocates its reproductive effort is part of its reproductive strategy. Effort can be expended in producing offspring, or caring for them, or both. Different strategies carry different costs and benefits.
- Of the total reproductive effort, the amount remaining after production of the offspring can be allocated to parental care. At one extreme, most invertebrates expend their total reproductive effort in producing eggs and sperm and there is no parental care. At the other extreme, mammals invest heavily in a small number of offspring and the parental care cost is substantial. Between this is a continuum, with some animals adopting alternative strategies, such as brood parasitism. No strategy is necessarily better than any other. They are different solutions to the problem of successful reproduction.

Many eggs or young produced

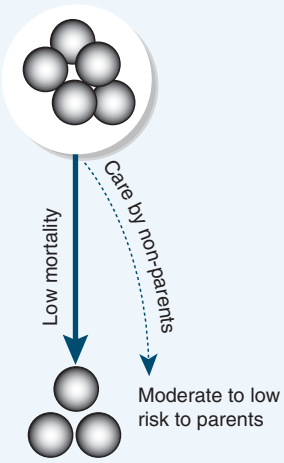


Low percentage of offspring survive to reproductive age

Little or no parental care

- Large number of offspring produced.
- Low reproductive effort per offspring.
- Little or no parental care of offspring.
- Reproductive effort is put into producing the offspring, not parental care.
- Examples: most fish, amphibians, reptiles, and invertebrates.

Moderate number of eggs produced

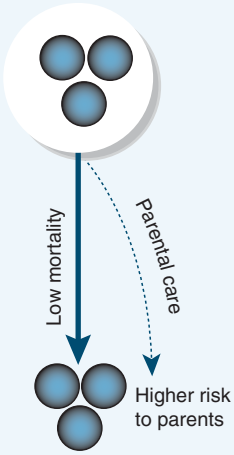


High percentage of offspring survive to reproductive age

Brood parasites

- Moderate number of offspring.
- Reproductive effort per offspring is moderate to low.
- Rely on others to raise offspring.
- Risk of egg loss is mitigated by distributing eggs amongst a number of hosts.
- Examples: some birds, insects, and fish.

Few eggs or young produced



High percentage of offspring survive to reproductive age

Parental care

- Few offspring.
- High reproductive effort per offspring.
- Moderate to substantial care of offspring.
- Large reproductive effort put into raising offspring to a less vulnerable stage.
- Examples: most birds and mammals, some fish.

4. Describe the different ways in which animals can allocate their total reproductive effort: _____

5. Animals with parental care protect the investment they have already made in offspring. Explain how factors in the environment (e.g. food resources and risks to young) might influence how much care is provided by each parent:

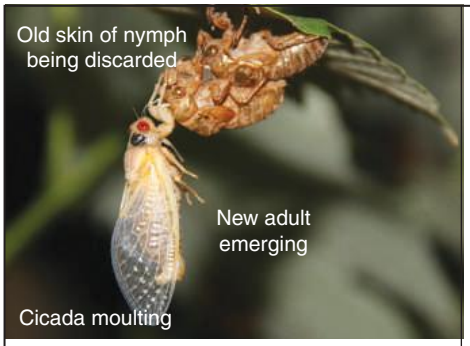
6. Why is no reproductive strategy necessarily better than any other? _____

ISBN: 978-1-98-856655-9

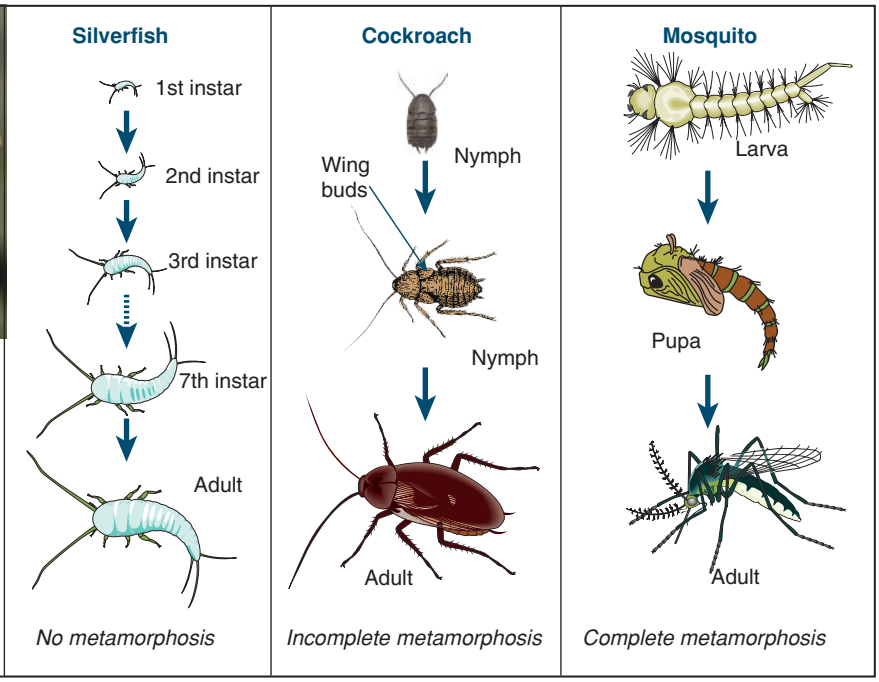
19 Insect Life Cycles

Key Idea: The life cycle of most insects involves metamorphosis from an immature form to an adult.

Insects possess an exoskeleton: a tough outer layer of chitin that must be moulted, or shed, to allow growth. The growth and development of most insects from an immature form into an adult is achieved by a process called metamorphosis. Most insects undergo a complete metamorphosis, which involves egg, larval, pupal, and adult stages. Other insects go through a process of incomplete metamorphosis, which involves several nymphal stages but no pupal stage. The term, instar is given to the developing nymph forms. In all insects, there may be several larval instars characterised by size, altered body proportions, or changes in body segments. In insects that undergo complete metamorphosis, the adult may have a different morphology and lifestyle from the larval stage.



The three distinct life cycle patterns seen in insects are shown on the right. Some, such as the primitive, wingless silverfish, exhibit little change with each moult. Insects from more primitive orders (e.g. cockroaches) hatch from eggs to resemble miniature adults, but with undeveloped reproductive organs and lacking wings. With each moult, their wings gradually increase in size and their reproductive organs develop. More advanced insects, e.g. butterflies and mosquitoes, exhibit egg, larva, pupa, and adult sequence.



The life cycle of the monarch butterfly *Danaus plexippus*

- The monarch butterfly has a life-cycle typical of an insect that undergoes complete metamorphosis. It passes through four completely different forms: a tiny egg, 5 stages of larval or caterpillar growth, a dormant pupa (chrysalis), and finally an adult butterfly.
- The whole growth process of the monarch takes a little over a month to complete in midsummer, longer at other, cooler, times of the year. Cold days slow down metabolism and therefore growth rate. The lifespan of the monarch has been estimated at 60-70 days. This lifespan becomes as much as 6 or 7 months if the butterfly emerges in the autumn and needs to hibernate through the winter, before egg-laying begins.


1. Two adults mating


2. Female laying eggs


3. Caterpillar larvae feeding


4. Chrysalis


5. An adult hatching

Stage in life cycle:	Egg	Larva	Pupa	Adult
Function:	Embryo	Growth	Reconstruction	Reproduction and dispersal
Instar:		1 2 3 4 5	6	7
Event:	*Oviposition*	*Hatching*	*Instar moults*	*Pupation*	*Emergence*	*Courtship*	*Egg laying*	*Death*

ISBN: 978-1-98-856655-9

1. List the stages in the following types of insect life cycles:

   (a) Complete metamorphosis: _____

   (b) Incomplete metamorphosis: _____

   (c) Growth and moulting: _____

2. Describe the purpose of each of the following stages in an insect life cycle exhibiting complete metamorphosis:

   (a) Egg: _____

   (b) Larva: _____

   (c) Pupa: _____

   (d) Adult: _____

3. Provide two examples of insects exhibiting each of the following types of life cycles:

   (a) Complete metamorphosis: _____

   (b) Incomplete metamorphosis: _____

   (c) Growth and moulting: _____

4. Describe one advantage of having a life cycle where the young undergo metamorphosis into the adult stage:

   _____

   _____

   _____

   _____

5. Identify a type of vertebrate that exhibits complete metamorphosis between the juvenile and adult stages:

   _____

6. Insects, unlike vertebrates, experience a problem when growing in size because they possess an exoskeleton.

   (a) Why does the exoskeleton create a problem for insect growth? _____

   _____

   (b) How do insects solve this problem? _____

   _____

7. Discuss the adaptive role of metamorphosis in the life cycles of insects, including reference to the exploitation of habitat and resources:

   _____

   _____

   _____

   _____

   _____

   _____

8. Why is the life cycle of the monarch butterfly considerably longer over winter (relative to summer) months?

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

20 Mammalian Reproduction

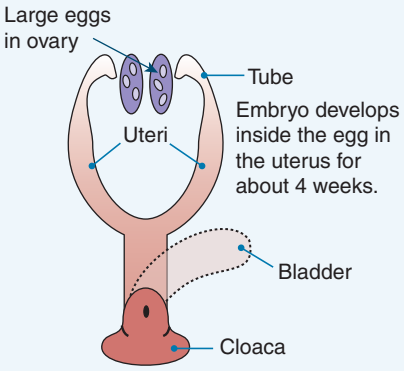
Key Idea: The three mammalian taxa have evolved different reproductive strategies with quite different energy allocations.

All mammals produce milk to feed their young. However, in other features of their reproduction and development, the three mammalian taxa (monotremes, marsupials, and placentals) are quite different. Monotremes, which are egg laying, are regarded as being the most primitive.

Monotremes

Examples: Platypus, Echidna

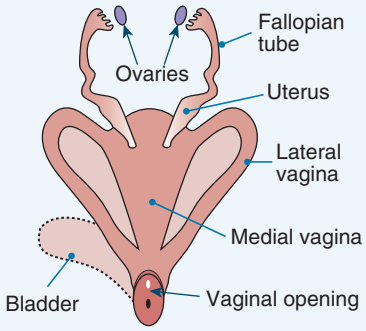
- Egg-laying habit (two at a time)
- Common cloaca (not separate anal and urine/genital openings)
- Large, yolky eggs
- Eggs incubated after laying
- After hatching, young lap milk from teatless mammary glands



Marsupials

Examples: Wallabies, Kangaroos, Wombats, Koala, Possums

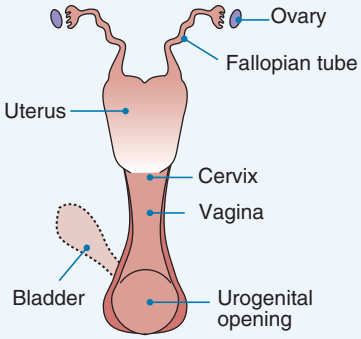
- At least two, sometimes three, vaginae
- Very short gestation of young in the uterus
- A very simple placenta is sometimes formed, but only for a very short time
- Tiny, embryo-like young at birth
- Development completed inside a pouch
- Young attach to teat in pouch to suckle



Placentals

Examples: Rodents, Primates

- Single common vagina and sometimes a common large uterus (e.g. humans)
- Eggs are small with very little yolk
- Placenta (complex embryonic-maternal connection) nourishes the embryo
- Young relatively well-developed at birth
- Young are suckled with milk after birth



1. Why are monotremes, marsupials, and placentals all classed as mammals? _____

_____

2. Why are monotremes regarded as being very primitive mammals? _____

_____

_____

3. Describe two features of marsupial reproduction that are advancements on the monotreme reproductive pattern:

_____

_____

_____

4. In what way is the reproductive plan in placentals more highly developed than that of marsupials?

_____

_____

_____

ISBN: 978-1-98-856655-9

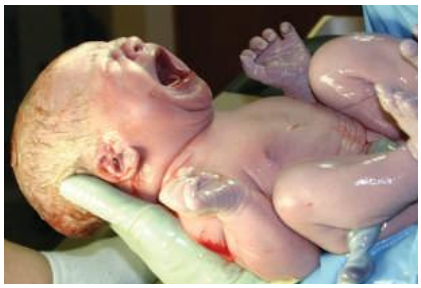
21 Gestational Development

Key Idea: There is some relationship between animal size and gestational period but other factors influence the degree of development and independence of newborn mammals.

The gestational period, or length of pregnancy, of mammals varies greatly. Although the gestational period is often longer for larger animals, factors other than size determine both length of pregnancy and level of development in the young at birth. These may include life-span, developmental rates, number of offspring produced, and threats (e.g. being eaten by other animals). Animals are classed as either precocial or altricial at birth (below), but there are varying degrees of classification between the two extremes.

Mammals which move independently soon after birth, and that see and hear well, are called **precocial**. Mobility is important, as their defence against predation is to run. Large hoofed grazers tend to be precocial.

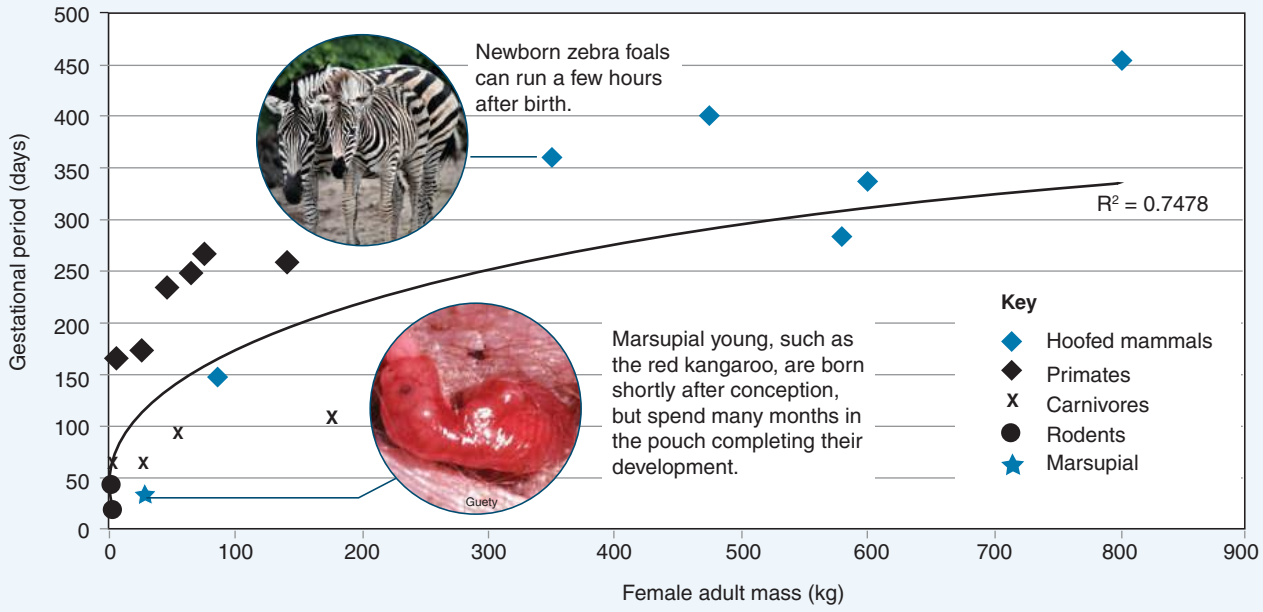
Altricial mammals are born relatively helpless. Often they are unable to see and are fairly immobile. The young of many species are born hairless, with their eyes shut. Rodents, cats, dogs, and marsupials are altricial species.



Ernest F cc3.0

Newborn primates show varying degrees of precocial or altricial features at birth. Many primates move about shortly after birth and their eyes are open. In contrast, newborn humans have long periods of dependency.

Gestational period versus female adult mass in mammals



1. Analyse the graph above and describe the relationship between animal size (weight) and gestational period:

_____

2. Suggest why hoofed mammals (sheep, zebra, horse cow, antelope) have long gestational periods: _____

_____

_____

_____

3. (a) What can you say about the position of the primates on the plot above? _____

_____

(b) Can you suggest a reason for this pattern? _____

_____

ISBN: 978-1-98-856655-9

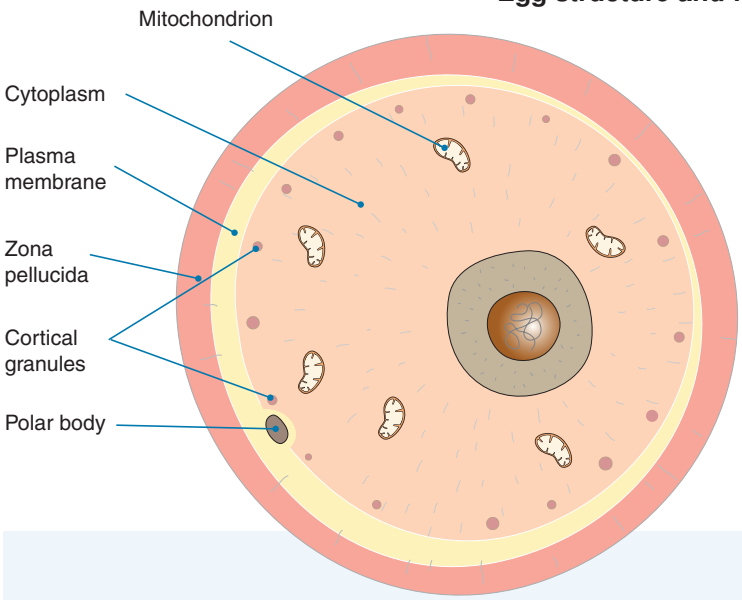
22 Gametes

Key Idea: Gametes are the sex cells of organisms. Male and female gametes differ in their size, shape, and number.

Gametes (sex cells) are produced for the purposes of sexual reproduction. The gametes of male and female mammals differ greatly in size, shape, and number. These differences reflect their different roles in fertilisation and reproduction.

Male gametes (**sperm**) are highly motile and produced in very large numbers throughout life. Female gametes (**eggs** or ova) are large, relatively few in number, and immobile. Eggs and sperm are produced by meiosis in a process called **gametogenesis**. Gametogenesis in males is called **spermatogenesis**. In females, it is called **oogenesis**.

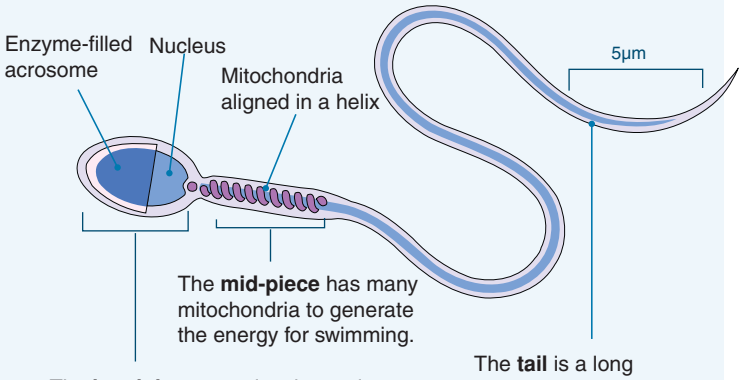
Egg structure and function



- The ovum is a simpler structure than the sperm cell. It has no propulsion mechanism and moves as a result of the wave-like motion of the ciliated cells lining the Fallopian tubes of the female reproductive tract. The ovum is required to survive for a much longer time than a sperm, so it contains many more nutrients and metabolites and, as a result, it is much larger than a sperm cell (up to 100µm).
- The contents of the ovum are similar to that of a typical mammalian cell, although it is externally surrounded by a jelly-like glycoprotein called the zona pellucida. A small polar body (the remnants of a sister cell) lies between the plasma membrane and zona pellucida. Cortical granules around the inner edge of the plasma membrane contain enzymes that are released once a sperm has penetrated the egg, forming a block to prevent further sperm entry (the cortical reaction).

Sperm structure and function

- Mature spermatozoa (sperm) are produced in the testes. Meiotic division of spermatocytes produces spermatids, which then differentiate into mature sperm.
- The sperm's structure reflects its purpose, which is to swim through the female reproductive tract to the ovum, penetrate the ovum's protective barrier, and donate its genetic material. A sperm cell comprises three regions: a headpiece, containing the nucleus and penetrative enzymes, an energy-producing mid-piece, and a tail for propulsion.
- Human sperm live for only about 48 hours, but they swim quickly and there are so many of them (millions per ejaculation) that usually some are able to reach the egg to fertilise it.



1. Why do sperm need to be motile? _____

_____

2. (a) How does an egg move along the Fallopian tube? _____

_____

(b) Why does a mature egg need to be so many times larger than a sperm? _____

_____

_____

3. Describe how each of the following three features of a sperm cell contribute to its role in reproduction:

(a) Tail: _____

(b) Mitochondria: _____

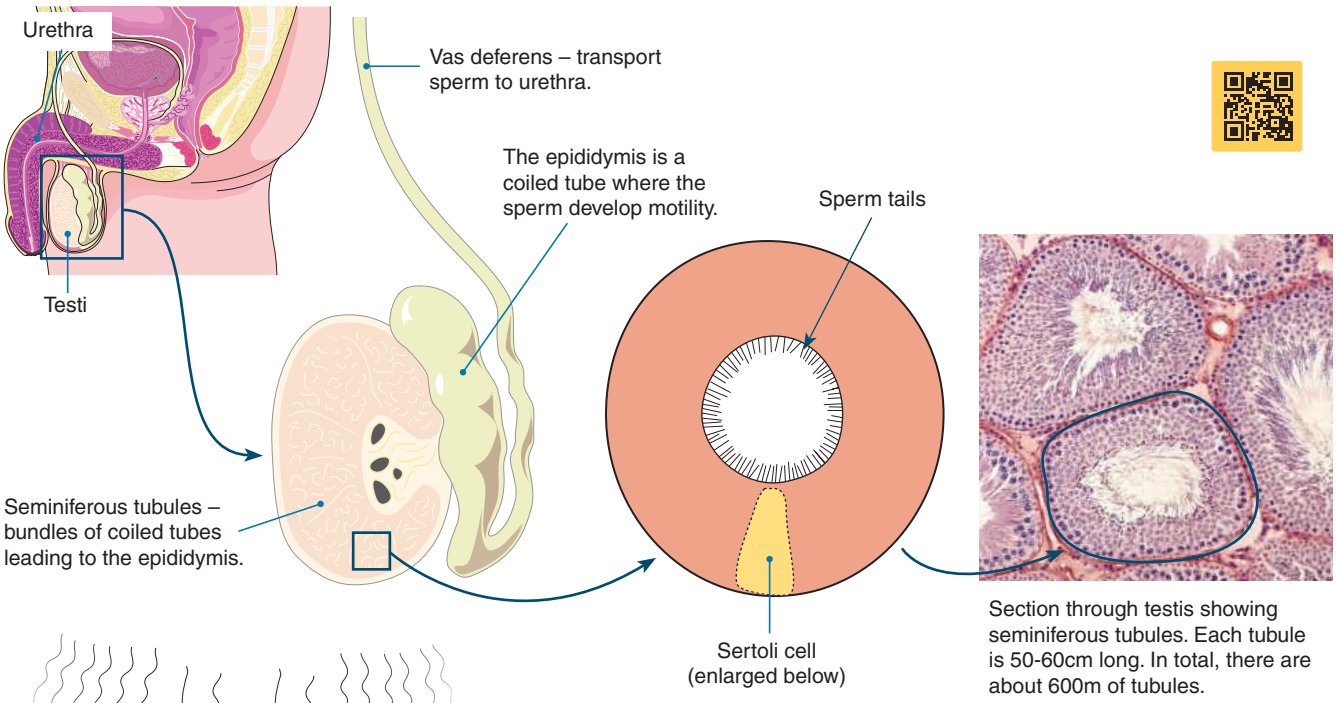
(c) Acrosome: _____

ISBN: 978-1-98-856655-9

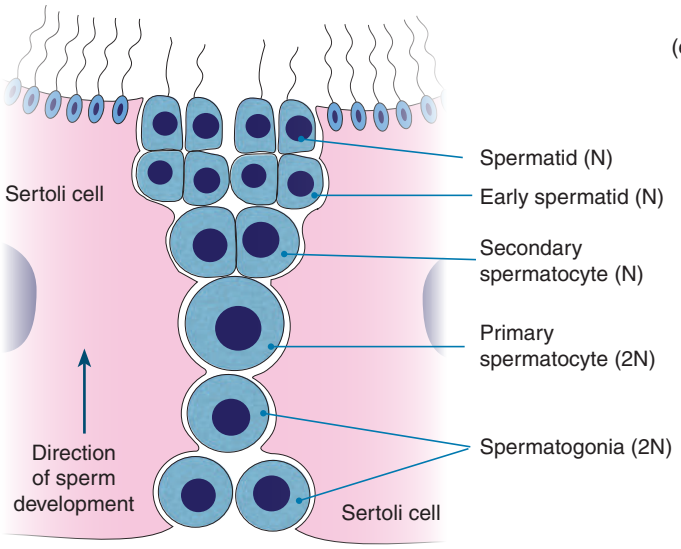
23 Spermatogenesis

Key Idea: Sperm is produced in the seminiferous tubules of the testes.

Sperm is produced via the process of spermatogenesis which occurs in the seminiferous tubules. Sperm production is a continual process. It takes 60-70 days for a sperm cell to fully develop, but several million sperm cells will be produced each day. A sperm cell will survive in the epididymis for about 3 weeks before being reabsorbed. When a sperm combines with an egg, it contributes half the genetic material of the offspring and, in mammals, determines its sex.



Section through testis showing seminiferous tubules. Each tubule is 50-60cm long. In total, there are about 600m of tubules.



Spermatogenesis

- Sperm (the male gametes) are produced by meiotic division of spermatogonia in the seminiferous tubules of the testes. The nucleus of the **germ cell** in the male divides twice to produce four similar-sized sperm cells. Spermatogenesis continues throughout an adult male's life.
- The developing sperm are nourished by supportive Sertoli cells. Sperm production occurs in response to the male steroid hormone, testosterone. Sperm formed in the testis enter the epididymis, where they mature and develop motility.

1. State the main role of the male reproductive system: _____

2. (a) Where does spermatogenesis occur? _____

   (b) What hormone controls this process? _____

   (c) From the diagrams above, what evidence do you have that spermatogenesis is most efficient at temperatures just below core body temperature?

3. Are sperm cells haploid or diploid? Explain: _____

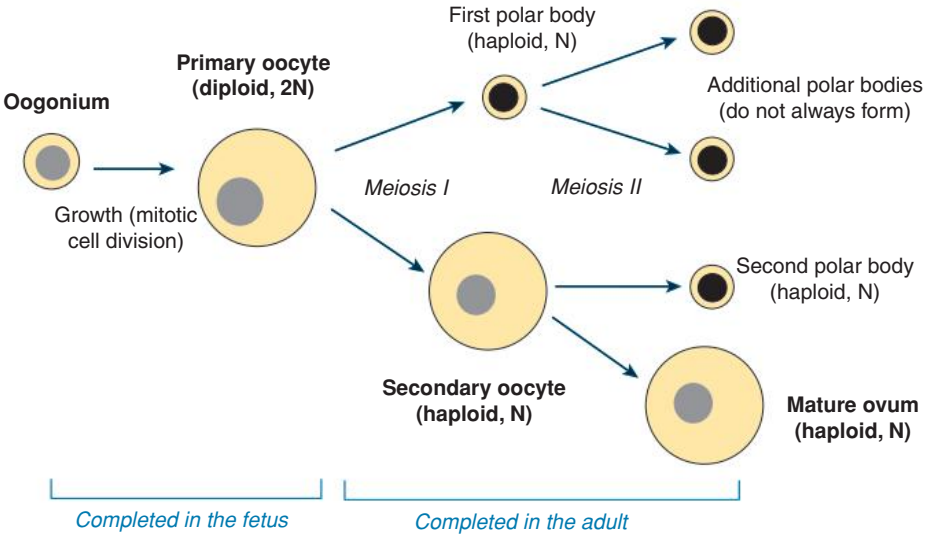
ISBN: 978-1-98-856655-9

24 Oogenesis

Key Idea: The ova are produced by a process called oogenesis which is only completed if the egg is fertilised.
The female reproductive system consists of the ovaries, Fallopian tubes, uterus, vagina, and external genitalia. Ova (egg) production initially begins in the embryo and is suspended until puberty. It resumes during a menstrual cycle, but is again suspended until fertilisation.

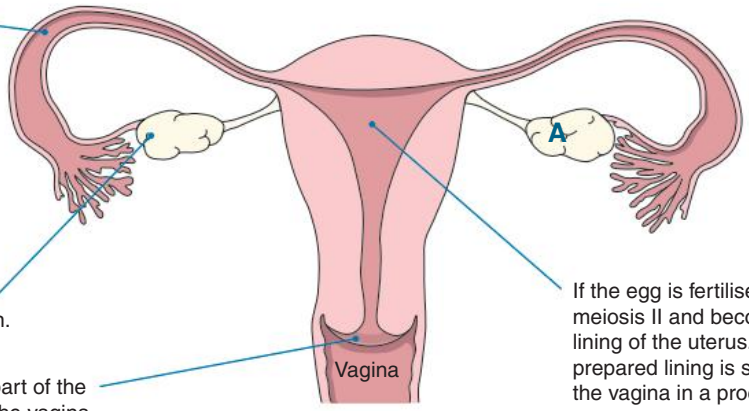
Oogenesis

- Unlike males, who produce sperm throughout their lifetime, females are born with their full complement of eggs and do not produce more. Oogenesis is the process by which mature **ova** are produced in the ovary. Mitosis in the oogonia of the female fetus produces diploid (2N) oocytes. These remain in a suspended state of the prophase stage of meiosis I until the female reaches puberty. At puberty, meiosis resumes. Each menstrual cycle, one or two ova resume development but, again, meiosis is suspended, this time in metaphase of meiosis II. The second meiotic division is only completed if the egg is fertilised.



Egg production and fertilisation

The unfertilised egg lives only for a day or so. It travels along the Fallopian tube, where fertilisation may occur, if sperm are present.



Eggs or ova are produced by the ovaries and are released at ovulation.

The cervix is the lower part of the uterus, connecting it to the vagina.

If the egg is fertilised, it will complete meiosis II and become implanted in the lining of the uterus. If it is not fertilised, the prepared lining is shed, passing out through the vagina in a process called menstruation.

Front view of uterus and associated structures

1. (a) Describe the difference between the lifetime production of gametes in males and females: _____

   _____

   _____

   (b) In humans, how might this affect reproductive decisions between males and females? _____

   _____

   _____

2. Describe the difference in the number of gametes produced from the original precursor cell between males and females:

   _____

   _____

3. What is the trigger for the completion of oogenesis? _____

4. Where does fertilisation occur? _____

ISBN: 978-1-98-856655-9

25 The Menstrual Cycle

Key Idea: The menstrual cycle involves cyclical changes in the ovaries and uterus to prepare for fertilisation of an egg.

In humans, fertilisation of the ovum (egg) is most likely to occur around the time of ovulation. The uterine lining (endometrium) thickens in preparation for pregnancy, but is shed as a bloody discharge through the vagina if fertilisation does not occur. This event, called **menstruation**, characterises the human reproductive or **menstrual cycle**. The menstrual cycle starts from the first day of bleeding and lasts for about 28 days. It involves predictable changes in response to pituitary and ovarian hormones and is divided into three phases (follicular, ovulatory, and luteal), defined by the events in each phase.

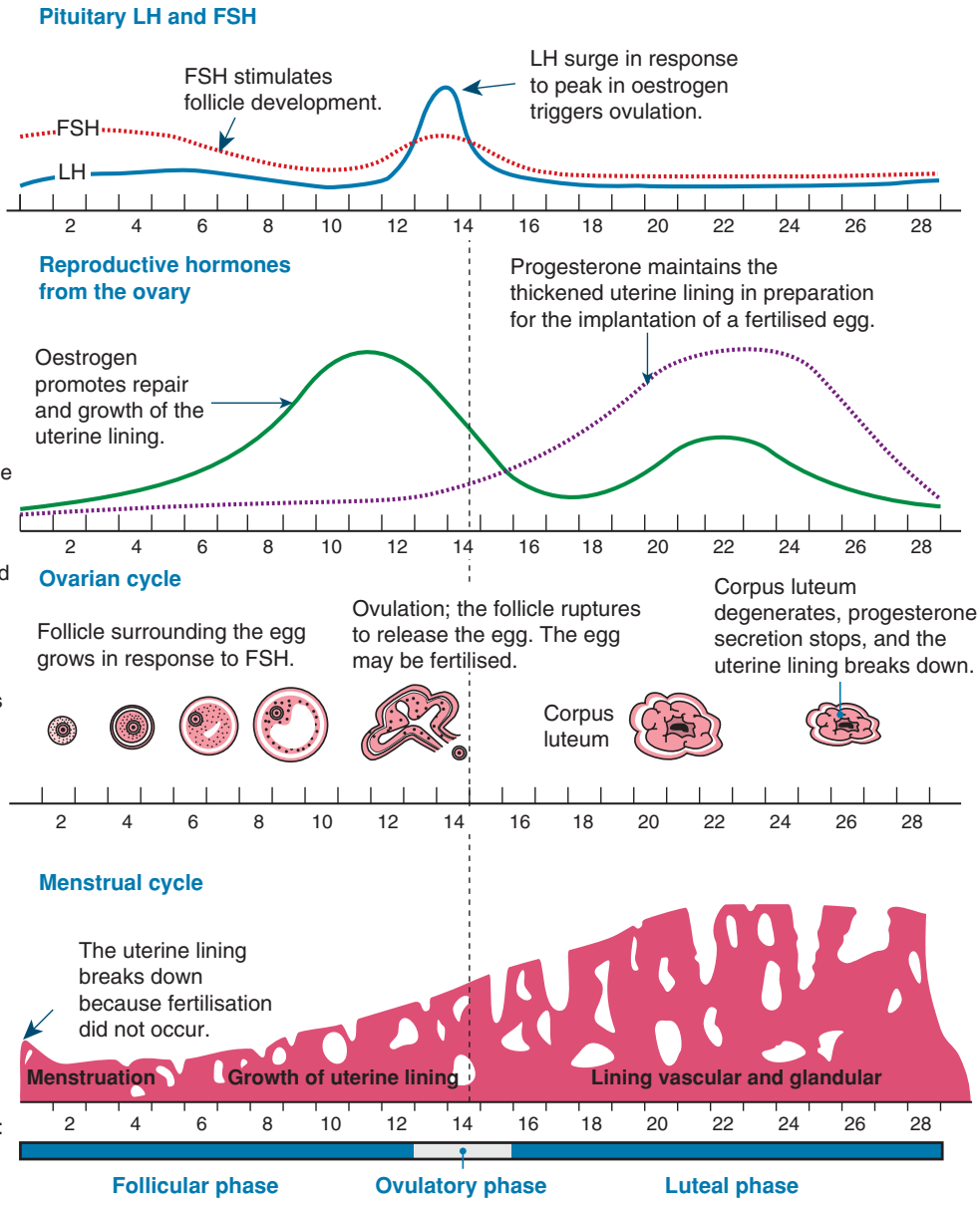
The menstrual cycle

Luteinising hormone (LH) and follicle stimulating hormone (FSH): FSH stimulates the development of the ovarian follicles, resulting in the release of oestrogen. Oestrogen levels peak, stimulating a surge in LH and triggering ovulation.

Hormone levels: One of the follicles that begins developing in response to FSH (the Graafian follicle) becomes dominant. In the first half of the cycle, oestrogen is secreted by this developing Graafian follicle. Later, the Graafian follicle develops into the corpus luteum (below right) which secretes large amounts of progesterone (and smaller amounts of oestrogen).

The corpus luteum: The Graafian follicle continues to grow and, at around day 14, ruptures to release the egg (ovulation). LH causes the ruptured follicle to develop into a corpus luteum (yellow body). The corpus luteum secretes progesterone which promotes full development of the uterine lining, maintains the embryo in the first 12 weeks of pregnancy, and inhibits the development of more follicles.

Menstruation: If fertilisation does not occur, the corpus luteum breaks down. Progesterone secretion declines, causing the uterine lining to be shed (menstruation). If fertilisation occurs, high progesterone levels maintain the thickened uterine lining. The placenta develops and nourishes the embryo completely by 12 weeks.



1. Identify the hormone responsible for:

   (a) Follicle growth: _____ (b) Ovulation: _____

2. Each month, several ovarian follicles begin development, but only one (the Graafian follicle) develops fully:

   (a) What hormone is secreted by the developing follicle? _____

   (b) What is the role of this hormone during the follicular phase? _____

   (c) What happens to the follicles that do not continue developing? _____

3. (a) What is the principal hormone secreted by the corpus luteum? _____

   (b) What is the purpose of this hormone? _____

4. What is the hormonal trigger for menstruation? _____

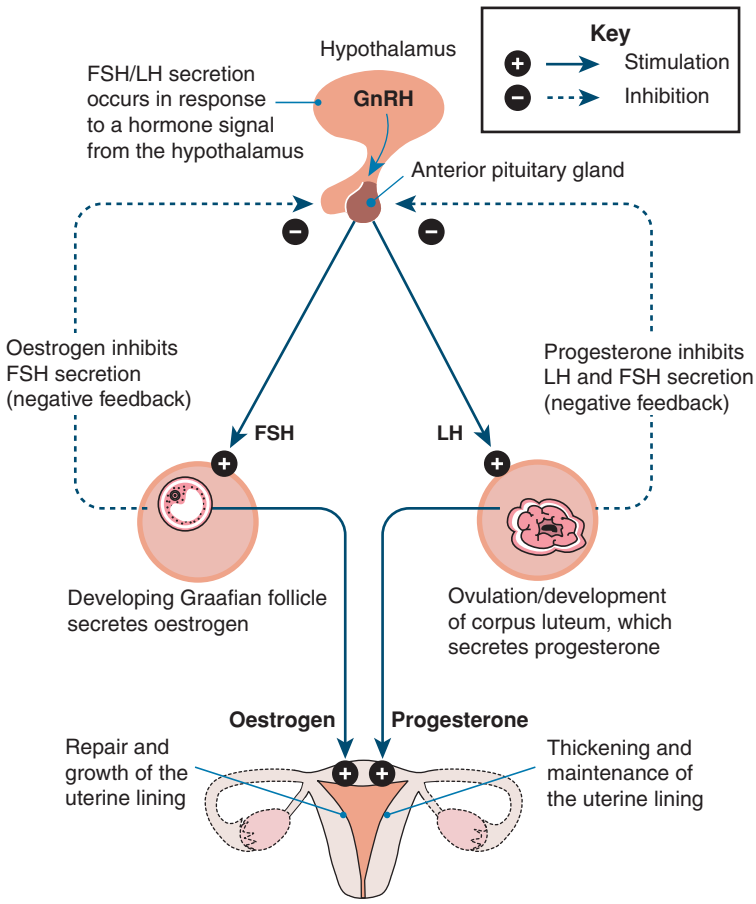
ISBN: 978-1-98-856655-9

26 Control of the Menstrual Cycle

Key Idea: Hormones from the hypothalamus and anterior pituitary regulate the menstrual cycle. The cycles of hormonal fluctuations can be manipulated to control fertility.

The female menstrual cycle is regulated by the interplay of several reproductive hormones. The main control centres for this regulation are the hypothalamus and the anterior pituitary gland. The hypothalamus secretes gonadotropin releasing hormone (GnRH), which is transported in capillaries to the anterior pituitary. Here, it induces the release of two hormones: follicle stimulating hormone (FSH) and luteinising hormone (LH). These two hormones bring about the cyclical changes in the ovaries and uterus. Hormone levels during the menstrual cycle are regulated through negative feedback mechanisms, except for the mid cycle LH surge. The normal regulation of the cycle can be manipulated with artificial hormones for the purposes of contraception.

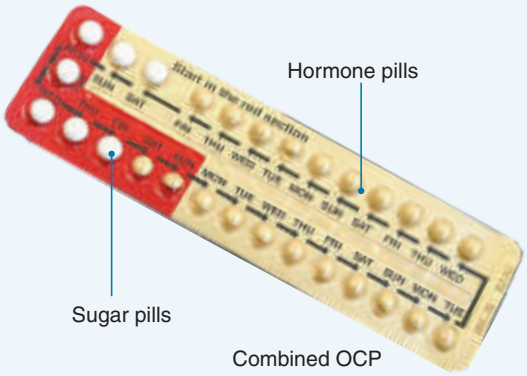
Control of the menstrual cycle



- **In the first half of the cycle**, FSH stimulates follicle development in the ovary. The developing follicle secretes oestrogen which acts on the uterus and, in the anterior pituitary, inhibits FSH secretion.
- **In the second half of the cycle**, LH induces ovulation and development of the corpus luteum. The corpus luteum secretes progesterone which acts on the uterus and also inhibits further secretion of LH (and also FSH).

Contraception

Contraception is used to prevent fertilisation of an egg by a sperm. Contraceptive methods include physical barriers, e.g. condoms, that prevent the egg and sperm meeting, or chemical interference with the menstrual cycle to inhibit egg production. This is done through oral contraceptives or hormone implants.



Hormonal contraception

The oral contraceptive pill (OCP) is a commonly used, reliable contraceptive method. Combined oral contraceptive pills exploit the feedback regulation of the menstrual cycle. They contain combinations of synthetic oestrogens and progesterone. They are taken daily for 21 days, and raise the levels of these hormones in the blood so that FSH secretion and the development of ova are inhibited. Sugar pills are taken for 7 days. This allows menstruation to occur but is not long enough to allow ova to develop.

A second type of contraception is a progesterone-only mini-pill. The pill works by thickening the cervical mucus and preventing endometrial thickening, but it does not prevent ovulation.

1. Describe the roles of FSH and LH in the control of the menstrual cycle: _____

2. How does negative feedback regulate the secretion of FSH and LH in the menstrual cycle? _____

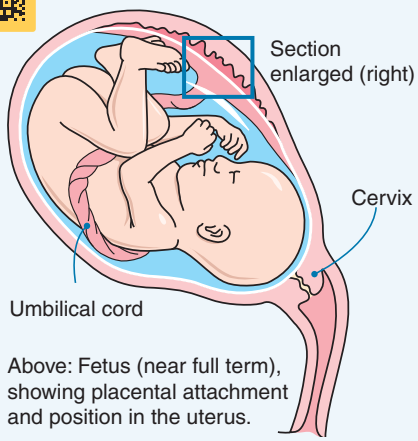
3. How does the OCP prevent conception (fertilisation of an egg by a sperm)? _____

ISBN: 978-1-98-856655-9

27 The Placenta

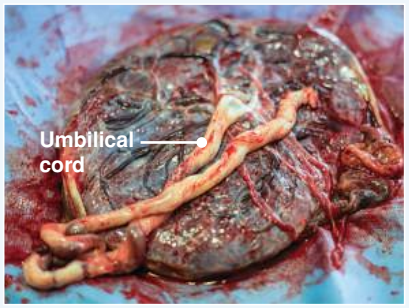
Key Idea: The placenta allows materials to be exchanged between the fetus and its mother. It also acts as a temporary endocrine organ, secreting hormones to maintain pregnancy.

The human fetus depends entirely on its mother for nutrients, oxygen, and the elimination of wastes. The **placenta** is the specialised organ that performs this role, enabling exchange between fetal and maternal tissues and allowing a prolonged period of fetal growth and development within the uterus. The placenta also has an endocrine role, producing progesterone and oestrogen to maintain the pregnancy.

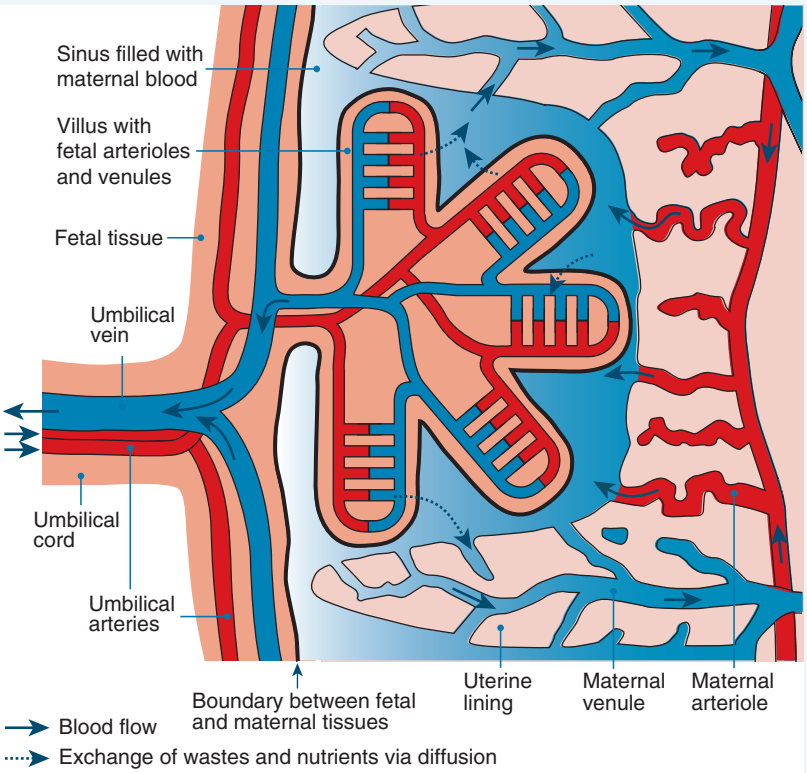


Above: Fetus (near full term), showing placental attachment and position in the uterus.

Below: Photograph of a human placenta, just after delivery.



Schematic diagram showing part of the placenta in section



The placenta is a disc-like organ, about the size of a dinner plate and weighing about 1kg. It develops when finger-like projections (villi) from the fetal membranes grow into the uterine lining. The villi contain the capillaries connecting the fetal arteries and vein. They continue invading the maternal tissue until they are bathed in the maternal blood sinuses. The maternal and fetal blood vessels are in such close proximity that oxygen and nutrients can diffuse from the maternal blood into the capillaries of the villi. From the villi, the nutrients circulate in the umbilical vein, returning to the fetal heart. Carbon dioxide and other wastes leave the fetus through the umbilical arteries, pass into the capillaries of the villi, and diffuse into the maternal blood. The fetal and maternal blood do not mix. The exchanges occur via diffusion through capillaries.

1. Describe the structure of the human placenta and explain its function: _____

2. The umbilical cord contains the fetal arteries and vein. Describe the status of the blood in each type of fetal vessel:

   (a) Fetal arteries: Oxygenated and containing nutrients / Deoxygenated and containing nitrogenous wastes (delete one).

   (b) Fetal vein: Oxygenated and containing nutrients / Deoxygenated and containing nitrogenous wastes (delete one).

3. Teratogens are substances that may cause malformations in embryonic development (e.g. nicotine, alcohol):

   (a) Why do substances ingested by the mother have the potential to be harmful to the fetus?

   (b) Why is cigarette smoking so harmful to fetal development? _____

ISBN: 978-1-98-856655-9

28 Fertilisation and Early Growth

Key Idea: The union of a male and a female gamete to form a zygote is called fertilisation. It involves several distinct stages.

Fertilisation occurs when a sperm penetrates an egg cell at the secondary oocyte stage. The sperm and egg nuclei then unite to form the zygote. In mammals, the entry of a sperm into the egg triggers specific mechanisms to prevent polyspermy (fertilisation of the egg by more than one sperm). These include a change in membrane potential, and the cortical reaction (see below). A zygote resulting from polyspermy contains too many chromosomes, and is not viable, i.e. it does not develop. Fertilisation is seen as time 0 in a period of gestation (pregnancy) and has five stages (below). After fertilisation, the zygote begins its development, i.e. its growth and differentiation into a multicellular organism.

Fertilisation (Time 0)

The stages in fertilisation are represented below in a numbered sequence (1-5)

1. Capacitation

The surface of the sperm cell undergoes changes that are essential to enable the acrosome reaction and sperm entry.

2. The acrosome reaction

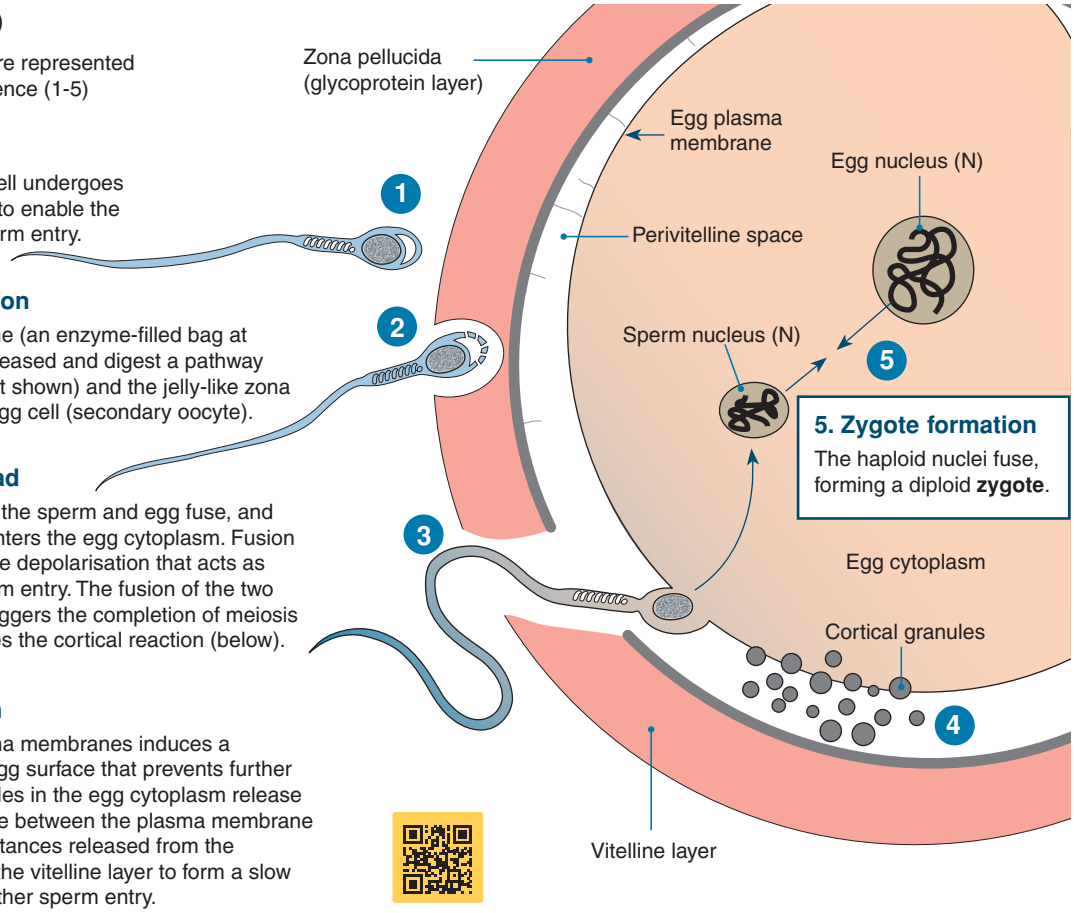
Enzymes from the acrosome (an enzyme-filled bag at the tip of the sperm) are released and digest a pathway through the follicle cells (not shown) and the jelly-like zona pellucida surrounding the egg cell (secondary oocyte).

3. Fusion of sperm head

The plasma membranes of the sperm and egg fuse, and the nucleus of the sperm enters the egg cytoplasm. Fusion causes a sudden membrane depolarisation that acts as a 'fast block' to further sperm entry. The fusion of the two plasma membranes also triggers the completion of meiosis II in the egg cell and induces the cortical reaction (below).

4. The cortical reaction

The fusion of the two plasma membranes induces a permanent change in the egg surface that prevents further sperm entry. Cortical granules in the egg cytoplasm release their contents into the space between the plasma membrane and the vitelline layer. Substances released from the granules raise and harden the vitelline layer to form a slow and permanent block to further sperm entry.



1. Briefly describe the significant events and their importance at each of the following stages of fertilisation:

   (a) Capacitation: _____

   _____

   (b) The acrosome reaction: _____

   _____

   (c) Fusion of egg and sperm plasma membranes: _____

   _____

   (d) The cortical reaction: _____

   _____

   (e) Fusion of egg and sperm nuclei: _____

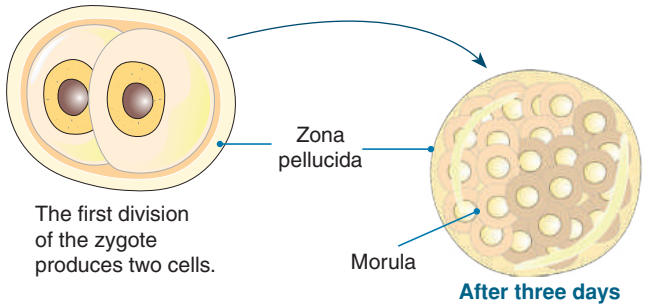
   _____

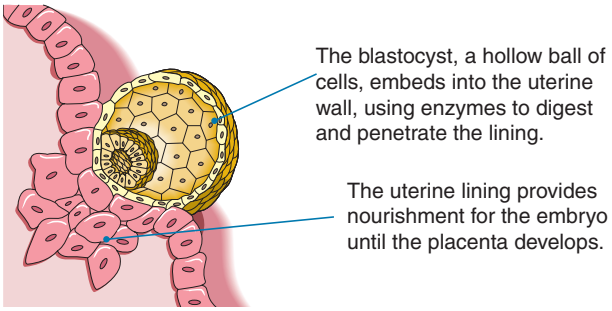
2. Why is it important that fertilisation of the egg by more than one sperm (polyspermy) does not occur? _____

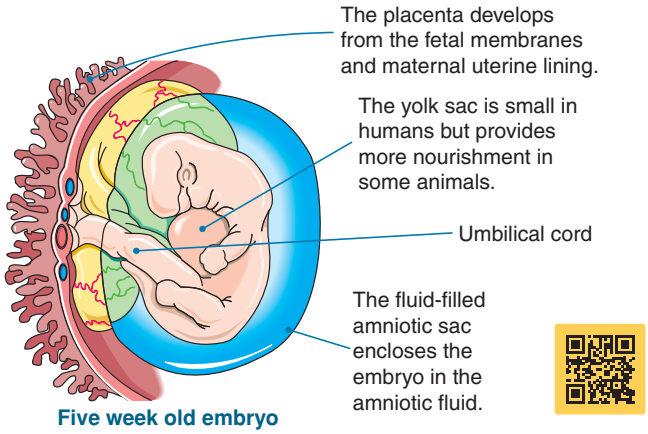
   _____

   _____

ISBN: 978-1-98-856655-9







Early growth and development

Cleavage, and the development of the morula

Immediately after fertilisation, rapid cell division takes place. These early cell divisions are called **cleavage** and they increase the number of cells, but not the size of the zygote. The first cleavage is completed after 36 hours, and each succeeding division takes less time. After 3 days, successive cleavages have produced a solid mass of cells called the **morula** (left), which is still about the same size as the original zygote.

Implantation of the blastocyst (after 6-8 days)

After several days in the uterus, the morula develops into the blastocyst. It makes contact with the uterine lining and pushes deeply into it, ensuring a close maternal-fetal contact. Blood vessels provide early nourishment as they are opened up by enzymes secreted by the blastocyst. The embryo produces **HCG** (human chorionic gonadotropin), which prevents degeneration of the corpus luteum and signals that the woman is pregnant.

The embryo at 5-8 weeks

Five weeks after fertilisation, the embryo is only 4-5 mm long, but already the central nervous system has developed and the heart is beating. The embryonic membranes have formed; the amnion encloses the embryo in a fluid-filled space, and the allanto-chorion forms the fetal portion of the placenta. From two months the embryo is called a fetus. It is still small (30-40 mm long), but the limbs are well formed and the bones are beginning to harden. The face has a flat, rather featureless appearance with the eyes far apart. Fetal movements have begun and brain development proceeds rapidly. The placenta is well developed, although not fully functional until 12 weeks. The umbilical cord, containing the fetal umbilical arteries and vein, connects fetus and mother.

3. (a) Explain why the egg cell, when released from the ovary, is termed a secondary oocyte: _____

_____

(b) At which stage is its meiotic division completed? _____

4. What contribution do the sperm and egg cell make to each of the following:

(a) The nucleus of the zygote? Sperm contribution: _____ Egg contribution: _____

(b) The cytoplasm of the zygote? Sperm contribution: _____ Egg contribution: _____

5. What is meant by cleavage? Explain its significance to the early development of the embryo:

_____

_____

6. (a) What is the importance of implantation to the early nourishment of the embryo? _____

_____

(b) What is the purpose of HCG production by the embryo? _____

_____

_____

7. Why is the fetus particularly prone to damage from drugs towards the end of the first trimester (2-3 months)?

_____

ISBN: 978-1-98-856655-9

29 The Hormones of Pregnancy

Key Idea: Hormones secreted during pregnancy maintain the pregnancy and prepare the body for birth.

In a non-pregnant adult human female, the levels of oestrogen and progesterone regulate the secretion of the pituitary hormones that control the ovarian cycle. Pregnancy interrupts this cycle and maintains the corpus luteum and the placenta as endocrine organs, with the specific role of maintaining the developing fetus during its development. During the last month of pregnancy, the hormone, oxytocin induces the uterine contractions that will expel the baby from the uterus.

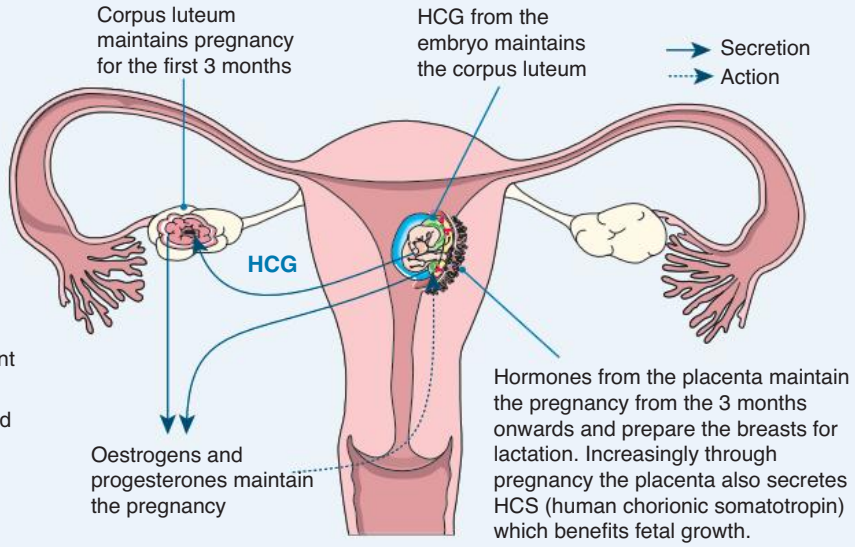
HCG (Human chorionic gonadotropin)
- Secreted by the developing embryo
- Maintains corpus luteum

Progesterone
- Maintains endometrium
- Inhibits uterine contraction

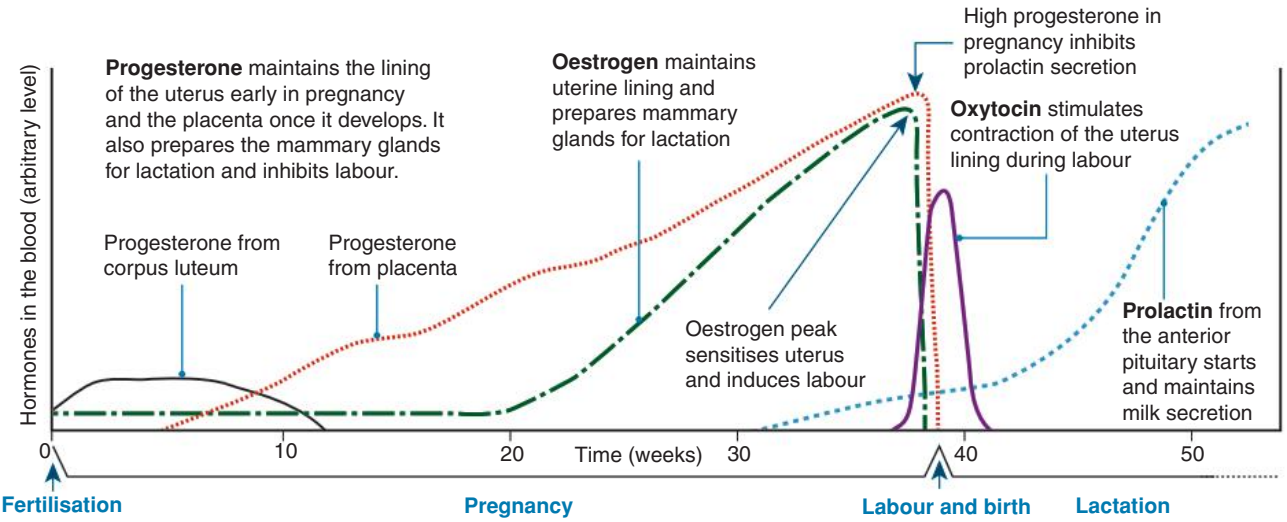
Oestrogens
- Maintain endometrium
- Prepare mammary glands for lactation
- High levels induce labour

Human placental lactogen (HPL)
- Stimulates breast growth and development

Relaxin
- Produced by the placenta towards the end of the pregnancy
- Relaxes pubic symphysis at birth
- Helps dilate cervix at birth



Hormonal changes during pregnancy, birth, and lactation



- During the first 12-16 weeks of pregnancy, the corpus luteum secretes enough progesterone to maintain the uterine lining and sustain the developing embryo. After this, the placenta takes over as the primary endocrine organ of pregnancy. Progesterone and oestrogen from the placenta maintain the uterine lining, stop further ova (eggs) developing, and prepare the breast tissue for lactation (milk production).
- At the end of pregnancy, the placenta begins to break down and progesterone levels fall. The uterus becomes sensitive to the high oestrogen levels, triggering labour to start. The oestrogen peak coincides with an increase in oxytocin, which stimulates uterine contractions in a positive feedback loop: the contractions and the increasing pressure on the cervix from the infant stimulate release of more oxytocin and more contractions, until the infant exits the birth canal.
- After birth, the secretion of prolactin increases. Prolactin maintains lactation during the period of infant nursing.

1. (a) Why is the corpus luteum the main source of progesterone in early pregnancy? _____

_____

(b) What hormones are responsible for maintaining pregnancy? _____

2. (a) Name two hormones involved in labour (onset of the birth process): _____

(b) Describe two physiological factors in initiating labour: _____

_____

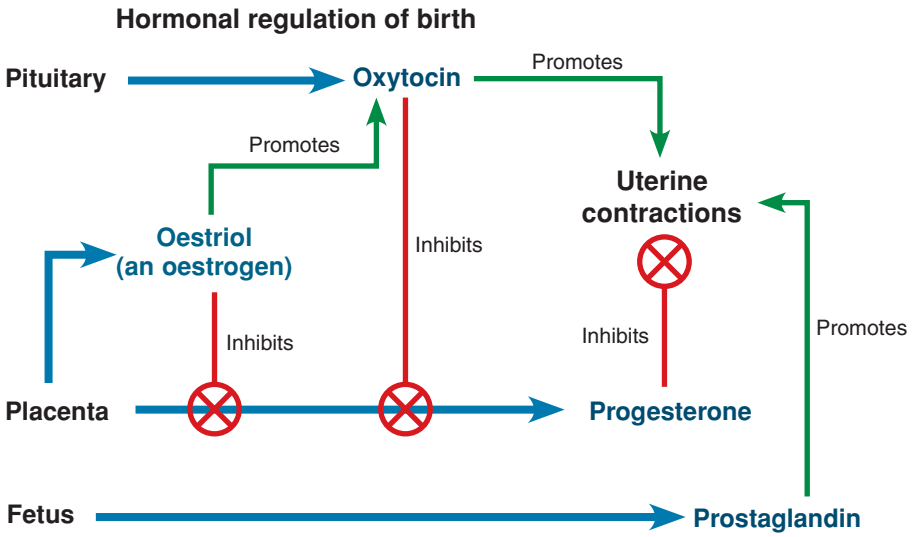
_____

ISBN: 978-1-98-856655-9

30 Birth and Lactation

Key Idea: Birth and lactation are both controlled by hormones and involve positive feedback.

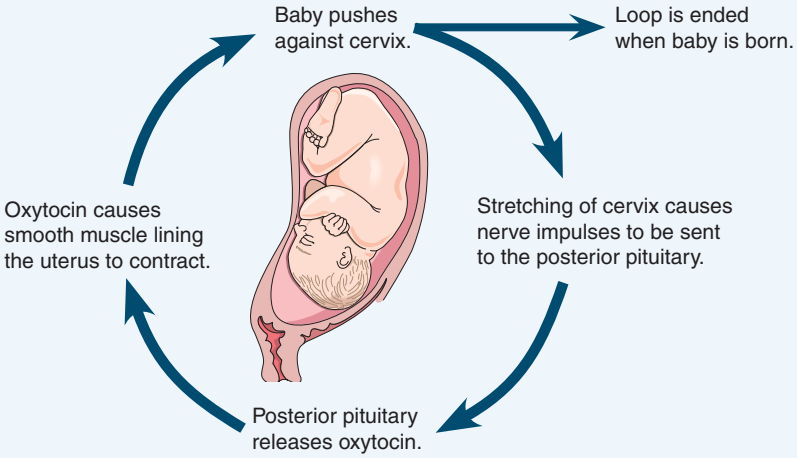
A human pregnancy lasts about 38 weeks after fertilisation. It ends in labour, the birth of the baby, and expulsion of the placenta. During pregnancy, progesterone maintains the placenta and inhibits contraction of the uterus. At the end of a pregnancy, increasing oestrogen induces labour. Prostaglandins, an ageing placenta, and the state of the fetus itself also play a role. At the same time, the hormone, oxytocin stimulates the contractions of the uterus that will expel the baby. After birth, the mother provides nutrition for the infant through lactation (milk production). Breast milk nourishes infants for the first 4-6 months of life. It also contains maternal antibodies, which protect the infant against infection.



- During the nine months of pregnancy, the placenta produces progesterone which inhibits uterine contractions. At the end of the pregnancy, the breakdown of the placenta results in a fall in the level of progesterone.
- The oxytocin produced by the placenta is then able to promote the production of oxytocin which promotes uterine contractions and begins the birth process.
- The fetus also begins to produce prostaglandin which further promotes uterine contractions. Uterine contractions produce a feedback loop that ends with the birth of the baby (below).

Positive feedback loop and birth

- Childbirth involves a positive feedback loop under hormonal control. A positive feedback loop reinforces a detected change.
- During childbirth, stretching of the cervix by the fetus triggers the release of hormones that enhance the birthing process by causing the contraction of the muscles lining the uterus.



1. (a) Which hormone is responsible for triggering the onset of labour? _____

   (b) What two other factors might influence the timing of labour onset? _____

   _____

   _____

2. Which two hormones inhibit progesterone? _____

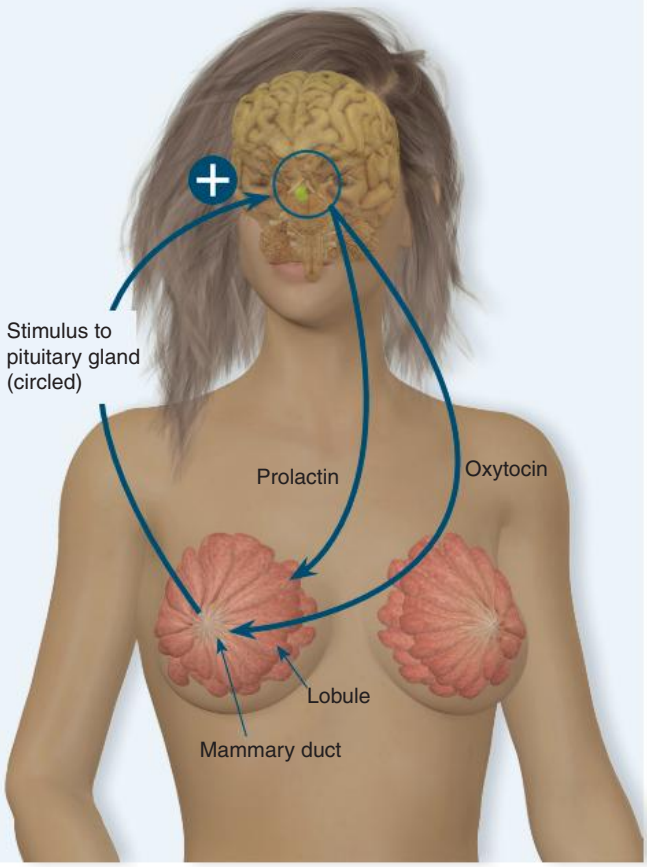
3. What causes the end of the birth feedback loop? _____

ISBN: 978-1-98-856655-9

Lactation and its control

- Lactation is the production and release of milk from mammary glands. After birth, levels of the hormone, prolactin increase sharply. Prolactin stimulates milk production in the alveoli, found within the lobules (right).
- Suckling maintains prolactin secretion and causes the release of oxytocin. Oxytocin induces the milk ducts to contract, resulting in milk release.
- The more an infant suckles, the more these hormones are produced. This is another example of positive feedback.

It is essential to establish breast feeding soon after birth, as this is when infants exhibit the strong reflexes that enable them to learn to suckle effectively. The first formed milk, colostrum, has very little sugar, virtually no fat, and is rich in maternal antibodies. Breast milk that is produced later has a higher fat content, and its composition varies as the nutritional needs of the infant change, during growth.



4. For each of the following processes, state the primary controlling hormone and its site of production:

   (a) Uterine contraction during labour: Hormone: _____ Site of production: _____

   (b) Production of milk: Hormone: _____ Site of production: _____

   (c) Milk ejection in response to suckling: Hormone: _____ Site of production: _____

5. Which hormone inhibits prolactin secretion during pregnancy? _____

6. Describe two benefits of breast feeding to the health of the infant:

   (a) _____

   (b) _____

7. (a) Describe the nutritional differences between the first formed milk (colostrum) and the milk that is produced later:

   _____

   _____

   (b) Suggest a reason for these differences: _____

   _____

8. Why would the nutritional composition of breast milk change during the lactation period from birth to six months?

   _____

9. Infants exhibit marked growth spurts at six weeks and three months of age. At these times, their energy intake requirements also increase sharply. With reference to what you know about the control of lactation, how could a breast-feeding mother continue to provide for the increased energy requirements of her infant?

   _____

   _____

ISBN: 978-1-98-856655-9

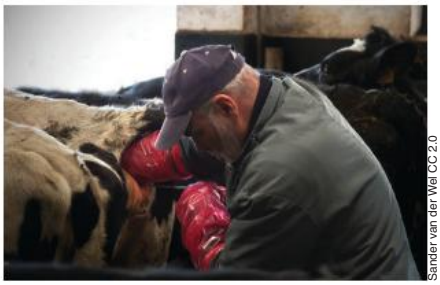
31 Manipulating Reproduction in Agriculture

Key Idea: The manipulation of reproduction in agriculture is an important technique for improving herd productivity.

Selective breeding is an important part of agriculture. Breeding males and females with specific characteristics produce desirable offspring that may have finer wool or increased milk production. **Artificial insemination** is used to quickly propagate useful characteristics across herds and is commonly used in cattle breeding. **Embryo splitting** is a technique by which cloned embryos are produced in the lab by splitting the embryo early in its development. The embryos are then implanted into surrogate mothers. Both techniques have their limits, as the physical characteristics of the offspring can never be completely predicted.

Artificial insemination

Artificial insemination is common in the production of quality dairy cattle. Sires are chosen based on desirable characteristics such as production of quality offspring.



Sander van der Wel CC 2.0

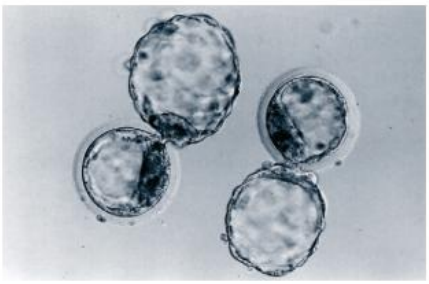
Breeding cows are inseminated. Semen from one sire can be used to inseminate sometimes thousands of cows without the bull having to travel to multiple farms.

By doing this, farmers can continually improve their stock quality and produce desired traits in offspring, such as lower methane production.

Cloning via embryo splitting

Stud merino rams, Australia

Livestock is selected for cloning on the basis of desirable qualities such as wool, meat, or milk productivity.



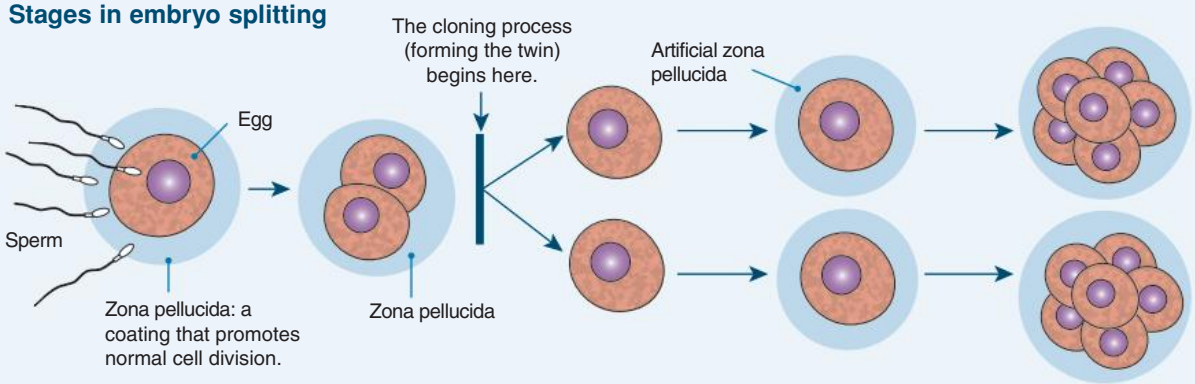
Dr David Wells, AgResearch

Cloned embryos immediately prior to implantation into a surrogate. These are at the blastocyst stage.

Twins, Pag Is. sheep, Croatia

The individuals produced by embryo splitting are identical to each but differ from the parents in the same way that twins do.

Stages in embryo splitting



Egg cells are removed from an animal and fertilised in a petri dish.

At the first stage of development, one of these fertilised eggs divides in two.

The zona pellucida is removed with an enzyme and the two cells are separated.

An artificial zona is added, allowing development to proceed.

The cells continue to divide, forming genetically identical embryos. These are implanted into surrogates.

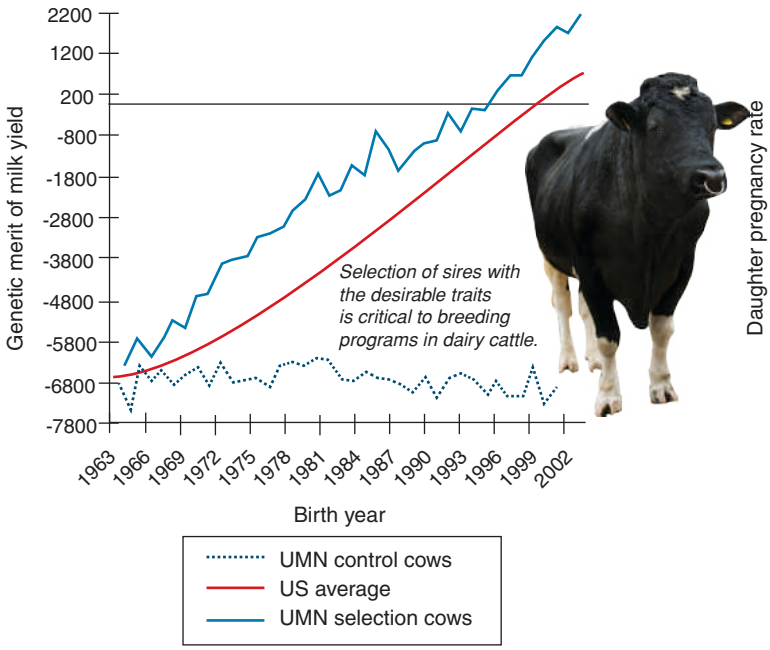
1. Why is artificial insemination commonly used in the agricultural sector, especially in the dairy industry?

ISBN: 978-1-98-856655-9

Improving livestock quality and production

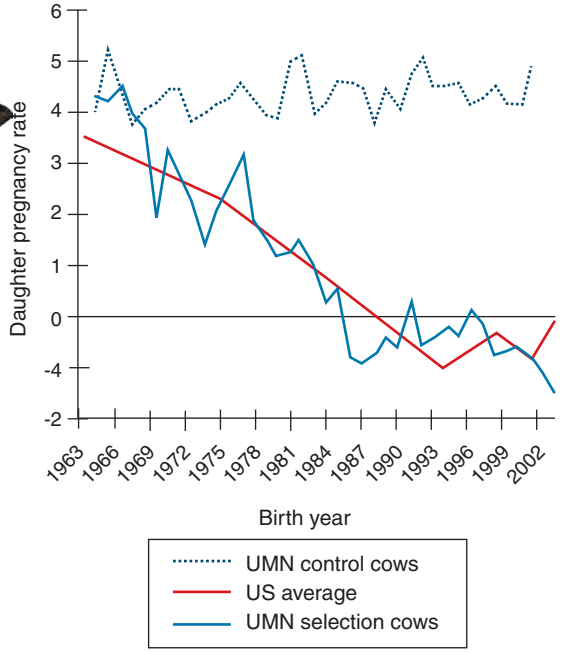
- Improving milk yield in cattle has been an important aim of the dairy industry for decades. Since the 1960s, the University of Minnesota has maintained a Holstein cattle herd in which there has been no selection. They also maintain a herd that was selected for increased milk production between 1965 and 1985.
- This makes it possible to easily compare the effects of artificial insemination and selective breeding on dairy cattle over the years.

Gain in genetic merit of milk yield



Milk production in the University of Minnesota (UMN) herd subjected to selective breeding increased in line with the US average production. In real terms, milk production per cow, per milking season, increased by 3740kg since 1964. The herd with no selection remained effectively constant for milk production.

Fertility in Holstein cows



Along with increased milk production there has been a distinct decrease in fertility. The fertility of the University of Minnesota (UMN) herd that was not subjected to selection remained constant, while the fertility of the herd selected for milk production decreased with the US fertility average.

Based on data from: USDA (2009) Breeding and Genetic Change in the Holstein Genome. Agricultural Research magazine

2. Why does embryo splitting not produce clones of the parents? _____

3. (a) Describe the relationship between milk yield and fertility in Holstein cows: _____

   (b) What does this suggest about where the genes for milk production and fertility are carried? _____

   (c) What limits might this place on maximum milk yield? _____

4. As a class, discuss what ethical concerns there may be around the use of artificial selection and artificial insemination in agriculture. Write a brief summary below:

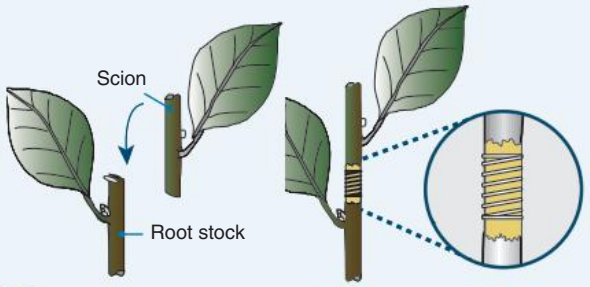
ISBN: 978-1-98-856655-9

32 Grafting

Key Idea: Grafting is the joining of the tissues of one plant to the tissues of another (usually a variety of the same species). Grafting is an important horticultural technique. It allows two or more different plants to be joined so that the combined plant has characteristics not fully present in either parent plant. Grafting is a way to produce a plant with a desirable suite of characteristics quickly, without needing to breed those features into a new variety. Plant breeders can focus on producing a plant which has a good rootstock without worrying about the fruit or leaves and *vice versa*. The superior fruiting plant can be grafted on to the superior rootstock, producing a plant with features of both.

Grafting

Grafting involves joining structures from two or more plants, usually varieties of the same species. Typically a twig section (**scion**) from one plant is joined to the shoot of another (**rootstock**). Grafting is a very common technique in the production of fruit and landscape trees.



1. A scion is prepared by taking a cutting. The scion is then grafted to another plant (rootstock).
2. The graft is covered in wax to prevent infection and held together with twine or raffia.

Multiple graftings

Multiple grafts can be made on to a single rootstock. A large tree can have virtually every branch grafted. Many people like to grow fruit trees but do not have the room to grow more than one or two. It is now possible to buy fruit trees with two, three, four, or more fruit types grafted onto the one plant. Grafting more than one plant variety together can also help pollination because many plants are not self fertile and need a pollinating plant nearby.

Bob Embleton

This apple tree has been grafted to produce two varieties. On the left, is a red skinned variety, whereas the right branches produce a green skinned variety.

Grafting process

A scion is removed from the parent plant prior to grafting.

Scion being grafted onto the stem of the rootstock.

The graft is sealed and covered to prevent water loss and infection.

The graft is then labelled for future reference and monitoring.

1. What is grafting? _____

2. Explain the difference between the scion and the rootstock: _____

3. Explain how grafting can be used to produce plants with dual qualities: _____

4. Explain why the grafting of multiple plant types can be useful: _____

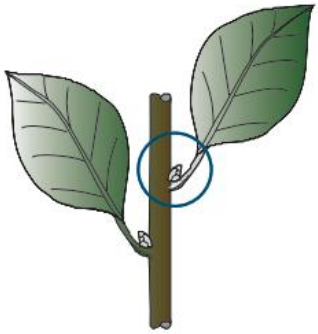
ISBN: 978-1-98-856655-9

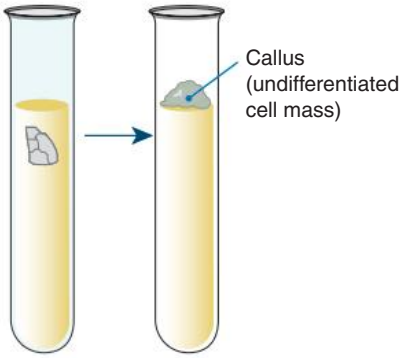
33 Micropropagation

Key Idea: Micropropagation is the propagation of multiple clones from one piece of plant tissue.

Micropropagation, by plant tissue culture, is a method used for cloning plants. It is widely used for fast production of large numbers of commercially important plant varieties with superior genotypes. It is also used in recovery programmes for endangered plant species. Micropropagation is possible because differentiated plant cells have the potential to give rise to all the cells of an adult plant (a property called **totipotency**). Micropopagation has many advantages over traditional methods of plant propagation but is very labour intensive. Its success is affected by factors such as the composition of the culture medium, selection of the original parent material, hormone levels, lighting, and temperature.

The process of micropropagation

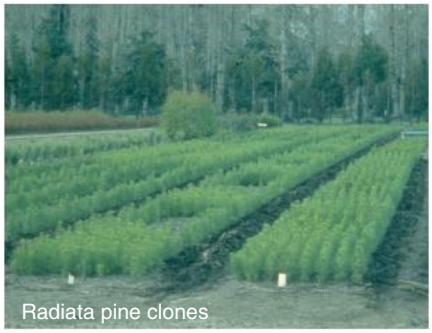




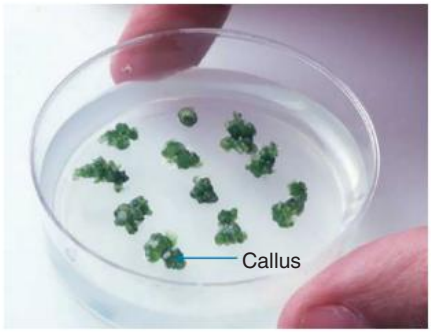
1 An **explant** (in this case an axial bud) is removed from a disease-free stock plant. Explants are commonly taken from cotyledons, axial buds, or roots.

2 Explant tissue is cultured in a sterile nutrient medium until a callus forms. The callus is transferred to another test tube containing growth hormones, and shoots are encouraged to grow.

3 Shoots develop from the callus and begin to photosynthesise. The new shoots are removed from the callus and placed in individual culture media. The process is repeated every few weeks so that one explant will give rise to many plants.



Micropropagation is extensively used in the forestry industry in many countries to produce uniform trees for timber.



Micropropagation is useful for quickly propagating genetically engineered plants, using material taken from a callus, containing genetically engineered material.

Joydeep

Banana plants, which are sterile, are produced by micropropagation, as they cannot be grown from seed.

1. What is the general purpose of micropropagation? _____

2. (a) Explain what a callus is: _____

   (b) How can a callus be stimulated to initiate root and shoot formation? _____

3. Describe some advantages and disadvantages of micropropagation: _____

ISBN: 978-1-98-856655-9

34 Case Study: Cloning in Horticulture

Key Idea: The use of cloning in horticulture has produced limited genetic diversity in some common fruit varieties.

Cloning has been used in horticulture for many hundreds of years. Many of the most commonly eaten fruits, including apples, oranges, grapes, and bananas are clones, produced by the propagation of one or two varieties for hundreds, or even thousands, of years. The cloning of fruit varieties has been extraordinarily successful in the mass production and distribution of plants and fruits. However, it has also created some problems. Many ancient varieties of fruit have been lost in the move to mass produce a small number of cultivars. Also, as many millions of plants are clones, they all have the same vulnerabilities, e.g. to disease. This makes it extremely important to preserve older plant varieties and maintain the genetic diversity necessary to produce vigorous, disease resistant varieties in the future.

Cloning and horticulture:

- Imagine you were walking through an orchard and found a fruit tree seedling that had been missed by the orchardist's mowing and spraying machines. You dig it up and plant it at home. When it fruits, you find it produces the best tasting, longest lasting fruit of its type ever.
- Wanting to capitalise on your discovery, you plant the seeds from one of the fruits, but they do not germinate. Closer inspection of seeds from other fruit on the tree finds the seeds never fully form. The tree cannot be regrown from seed; it is a one-off chance event, and sterile.
- However, there is another way to produce more of this unique fruit tree – cloning it, using cuttings and grafting. Some cuttings are grown directly into trees, others are grafted on to the rootstock of another variety of the fruit. Soon, thousands of new trees are produced and distributed to orchards. Within a few years, your new fruit variety is the dominant one grown worldwide. Within decades it is virtually the only fruit of that type grown.
- Then disaster strikes. The fruit is susceptible to a soil fungus found in a country different from the variety's origin. Unknowingly, the fungus has been spread worldwide in orchard equipment. Within a year of discovering the fungus, half of the trees are failing. Within two years, three quarters of the fruit trees worldwide are dead. Another year, and it is no longer economical to even plant the variety as the plants never reach maturity. Meanwhile, as a result of the fruit's dominance, nothing is left to replace it. For years, there is a massive shortage of the fruit, until older varieties can be re-established.
- Sound fictional? It has happened at least once and may not be far from happening again.

Zwifree cc 4.0

The Gros Michel (Big Mike) banana, now commercially extinct. Its physical properties (thick skin and dense bunches) made it ideal for export and it had a high concentration of the ester used for the 'banana' food flavouring. Vast areas of banana plantations succumbed to a fungus and exports stopped in the 1960s.

1. Why can't crops such as bananas and navel oranges be grown from seed? _____

2. Why are Granny Smith apples not produced from seed? _____

3. How do bananas illustrate the vulnerability of these crops? _____

4. Bananas are cultivated using micropropagation. What are some disadvantages of micropropagation for a vulnerable food plant like the banana?

ISBN: 978-1-98-856655-9

Cavendish banana

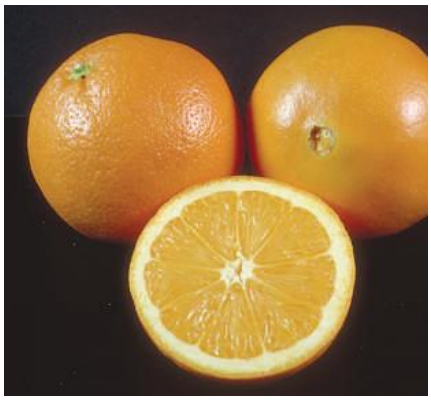
Young Cavendish clones under cultivation

There are many varieties of banana. All commercial bananas are descendents of the two banana species *Musa acuminata* and *M. balbisiana*. The most common banana, the Cavendish, is a triploid (three sets of chromosomes) variant of the banana species *Musa acuminata*. This makes it sterile. All the Cavendish plants worldwide are clones. The Cavendish replaced the Gros Michel, which was devastated by Panama disease, around the 1950s. The Cavendish is now being badly affected by a new strain of the disease and scientists are trying to find, or develop, a variety to replace the Cavendish, should the need arise.

Granny Smith variety

Granny Smith graft

The Granny Smith apple originated in Eastwood, New South Wales, around 1870. It is named after its discoverer, Maria Ann Smith, who also originally propagated it. How exactly she found the original tree is unclear, but it may have come from a variety of crab apple. The Granny Smith became extremely popular during WWII due to its long shelf life, making it easy to export. It is not grown commercially from seed as the fruit produced varies and is usually very tart/sour in taste. As a result, all the trees are clones, directly related to the original tree in Eastwood. It is still in the top twenty most popular apples in the USA.



The popular seedless navel orange

Navel orange tree grafted on to rootstock

In 1820, a mutation was found in an orange tree in Bahia, Brazil. The mutant tree produced a sweet, seedless fruit with an undeveloped fruit near the base, giving the characteristic 'navel'. Cuttings were grafted onto citrus root stock and new plants were grown. The new plants were taken to the United States and grown in California. The fruit was received well by orchardists and is now the single most popular orange variety in the world. However, because it is seedless, all the plants are clones grown by grafting onto root stock. Since its discovery, there have been a few new mutations that have produced new varieties of navel orange. Other new fruit varieties arising as a result of chance mutation are nectarines (from peaches), and red Anjou pears.

5. Occasionally, banana plants under cultivation will produce a 'sport'. This is a branch arising from a chance mutation that has different characteristics to the parent (e.g. in leaves or fruit).

   (a) Explain how this sport could be propagated to produce a new variety of banana:

   _____

   _____

   _____

   (b) How could this help towards ensuring the continued availability of bananas? _____

   _____

   _____

   _____

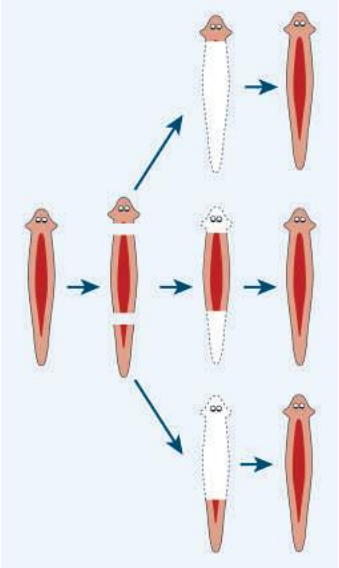
   (c) In what other crop plants has mutation helped to produce new varieties? _____

   _____

ISBN: 978-1-98-856655-9

35 Chapter Review: Did You Get It?

1. (a) What method of asexual reproduction is depicted in the diagram (right) and what does it involve?



(b) How is this process different from fission, in eukaryotic organisms? _____

(c) How is it different from budding? _____

2. Distinguish between grafting, and taking cuttings from plants: _____

3. Explain why the grafting of multiple plant types can be useful: _____

4. Explain how micropropagation is used in the production of new plants, and list its advantages and disadvantages:

5. (a) Describe an advantage of sexual reproduction, compared to asexual reproduction: _____

(b) Describe a disadvantage of sexual reproduction, compared to asexual reproduction: _____

6. Describe the benefits and costs of the internal fertilisation and development of the offspring (within the mother):

ISBN: 978-1-98-856655-9

CHAPTER 2

Cell Replication

Key terms

chromosome
crossing over
cytokinesis
diploid
DNA
DNA polymerase
gene
haploid
independent assortment
interphase
meiosis
mitosis
mutation
nucleic acid
nucleotide
RNA
semi-conservative replication

Inquiry question: *How important is it for genetic material to be replicated exactly?*

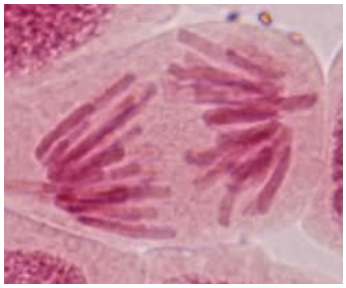
Activity number

Cell division

Key skills and knowledge

☐	1	Describe the various roles of cell division in the life cycles of different organisms, including reproduction, growth, and repair and replacement of tissues. Compare and contrast mitotic cell division and meiotic cell division.	36 37
☐	2	Describe the cell cycle in eukaryotes, including reference to DNA replication (S), growth (G_1 and G_2), and mitosis (M phase).	38
☐	3	Describe and explain cytokinesis in both plant cells and animal cells.	39
☐	4	Describe and model the events in each of the main stages of mitosis: prophase, metaphase, anaphase, and telophase.	40
☐	5	PRAC Modelling mitosis	41
☐	6	Describe the events in each of the main stages of meiosis: prophase I, metaphase I, anaphase I, telophase I, and prophase II, metaphase II, anaphase II, telophase II.	42
☐	7	Explain the importance of crossing over in meiosis in producing variation in gametes. Model the processes of meiosis including homologous chromosomes, crossing over, production of gametes, and fertilisation.	42 43
☐	8	PRAC Modelling meiosis using ice-block sticks	43

Brocken Inaglory



DNA replication

Key skills and knowledge

☐	9	Describe the structure of DNA and explain how its structure provides a mechanism for self-replication. Include reference to the base-pairing rule, the anti-parallel strands, and the role of hydrogen bonding between purines and pyrimidines.	44 49
☐	10	Describe the structure of the three types of RNA (mRNA, tRNA, and rRNA) and their functional roles in cellular activities. Compare and contrast RNA and DNA.	45
☐	11	PRAC Investigate the nature of DNA by making a DNA extraction.	46
☐	12	PRAC Create a model of DNA to demonstrate the base pairing rule.	47
☐	13	Analyse the evidence for the structure of DNA.	48
☐	14	Model Meselson and Stahl's investigation into the semi-conservative replication of DNA based on the Watson and Crick model of DNA.	50
☐	15	Explain the importance of mitosis and meiosis on the continuity of species.	51

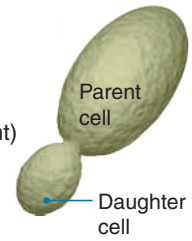
36 Why Cells Need to Divide

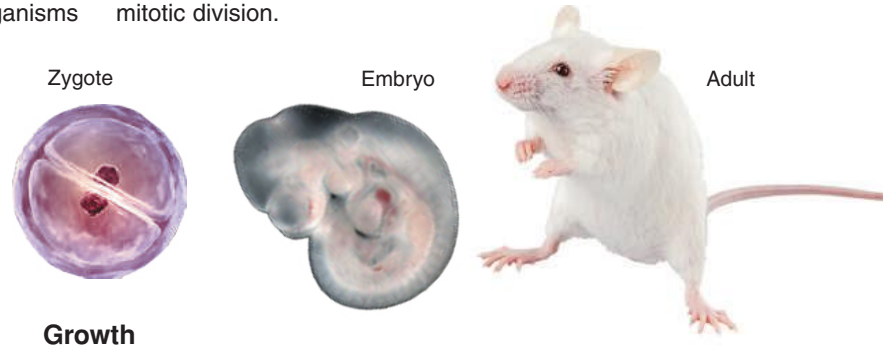
Key Idea: Mitotic cell division has three primary functions: growth of the organism, replacement of damaged or old cells, and, in some organisms, asexual reproduction.

Mitotic cell division produces daughter cells that are genetically identical to the parent cell. It has three purposes: growth, repair, and reproduction. Multicellular organisms grow from a single fertilised cell into a mature organism that may consist of several thousand to several trillion cells. Repair occurs by replacing damaged and old cells with new cells. Some unicellular eukaryotes such as yeasts, and some multicellular organisms, e.g. *Hydra*, reproduce asexually by mitotic division.

Asexual reproduction

Some simple eukaryotic organisms reproduce asexually by cell division. Yeasts, such as baker's yeast, can reproduce by budding. The parent cell buds to form a daughter cell (right) which eventually separates from the parent cell. Prokaryotes divide by binary fission, a different but superficially similar process.



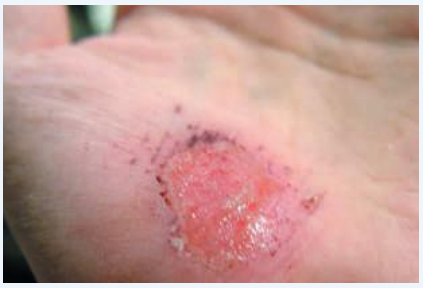


Growth

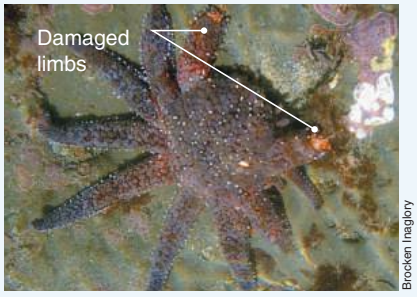
Multicellular organisms develop from a single fertilised egg cell (zygote) and grow by increasing the number of cells. Cells complete a cycle in which the cell copies its DNA, and then divides to produce two identical cells. During the period of growth, the rate of cell production is higher than the rate of cell deaths. Organisms, such as the 12 day old mouse embryo (above, centre), grow by increasing their total cell number and the cells become specialised as part of development.

Cell growth is highly regulated. Once the mouse reaches its adult size (above, right), physical growth stops and the number of cell deaths equals the number of new cells produced.

Bill Rhodes



Jpbarrass



Brocken Inaglory

Repair of damaged tissues

Mitotic cell division is responsible for the repair and replacement of damaged cells in multicellular organisms. When you break a bone or graze your skin, new cells are generated to repair the damage. Some organisms, like the sea star (above right), are able to generate new limbs if they are broken off.

1. Use examples to explain the role of cell division in:

(a) Growth of an organism: _____

_____

_____

_____

(b) Replacement of damaged cells: _____

_____

_____

_____

(c) Asexual reproduction: _____

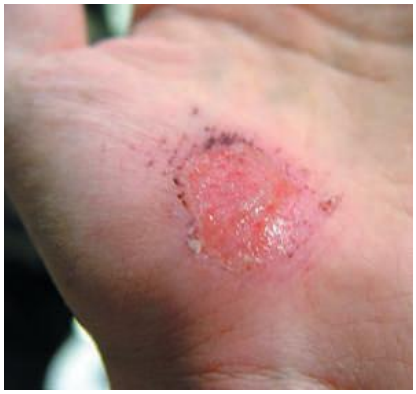
_____

_____

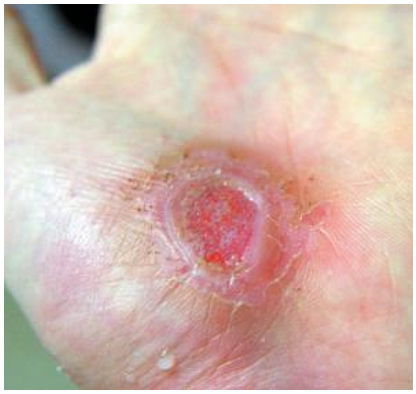
ISBN: 978-1-98-856655-9

Wounded!

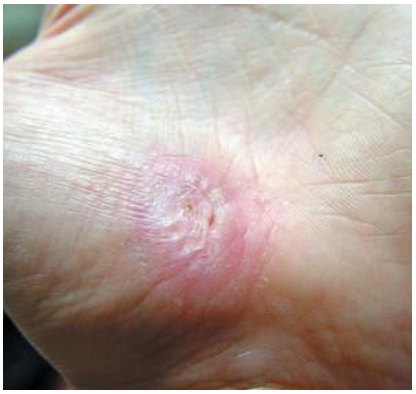
- The photos below show a wound soon after it occurred, 12 days later, and 21 days later.



1 day after



12 days after



21 days after

All photos Jpbarrass

2. (a) Looking at the images above, which part of the wound appears to heal first? _____

_____

(b) Suggest what is happening to the growth and division rate of the skin cells during the time the wound is healing:

_____

_____

3. The data below relates to the healing of the wound on the hand shown above. It shows the approximate area of the unhealed region of the wound over time:

Time (days)	Area unhealed (mm^2)
0.02	350
0.66	350
1	340
2	252
12	171
13	135
17	72
18	64
21	16
30	0

(a) Plot the data on the grid provided:

(b) Describe the trend in the wound size as shown by the data:

_____

_____

_____

4. What type of cell division produces the skin cells to heal the wound above? Comment on the genetic make up of the cells:

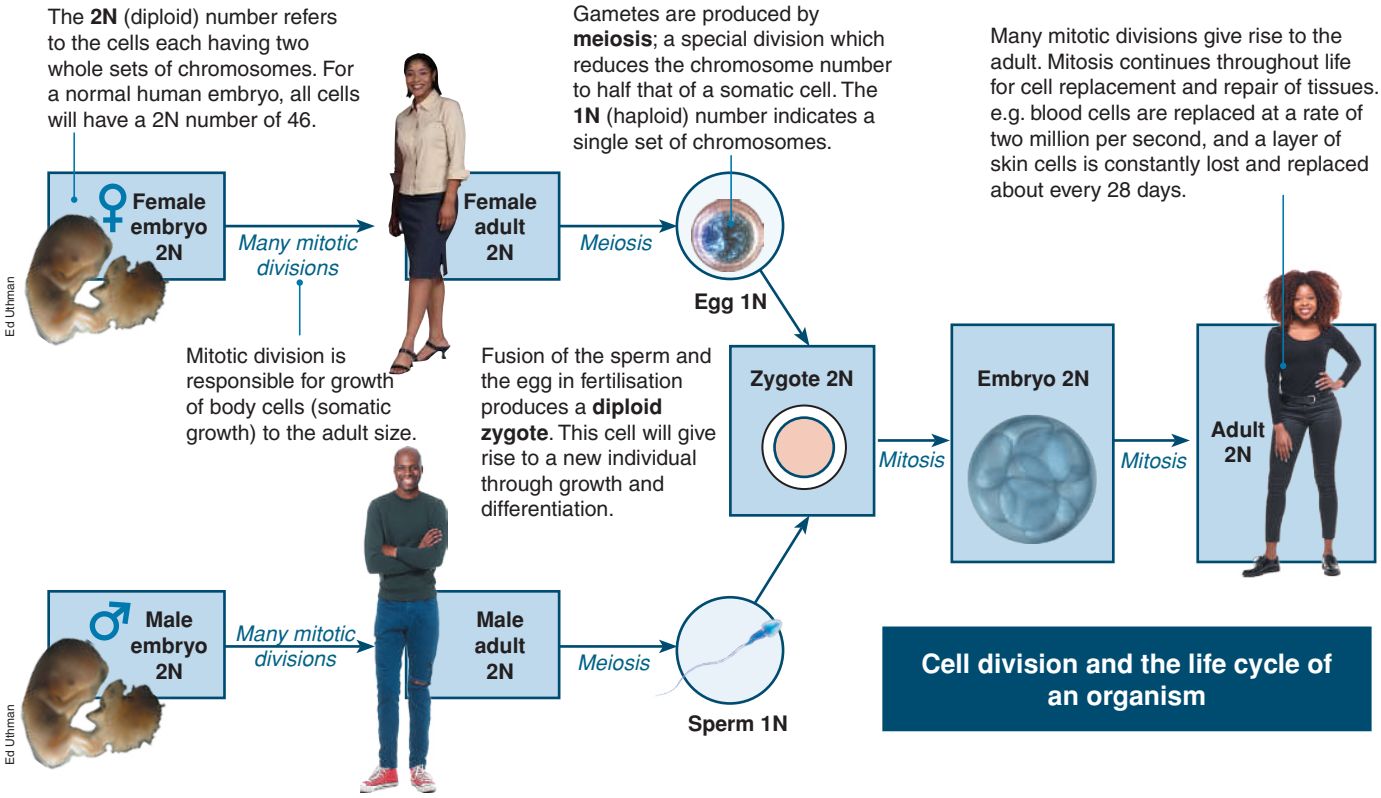
_____

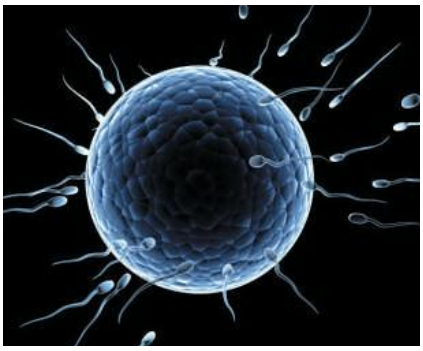
ISBN: 978-1-98-856655-9

37 Cell Division

Key Idea: There are two types of cell division in eukaryotes, mitosis and meiosis, but only meiosis produces cells that are genetically different to the parent cell.

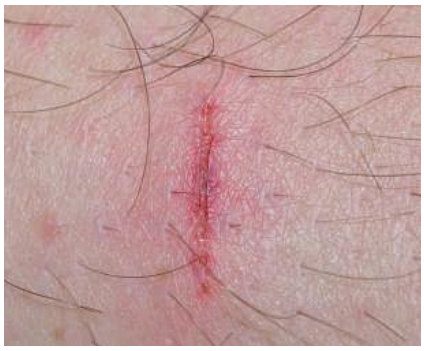
New cells are formed when existing cells divide. There are two forms of cell division in eukaryotes, mitosis and meiosis. **Mitosis** produces two identical daughter cells from a parent cell. **Meiosis** is a special type of cell division, it produces sex cells (gametes or spores) for sexual reproduction. In sexual reproduction, sex cells from two parents combine to form a new individual that is genetically different to its parents.





The sex cells in humans, called eggs and sperm, are produced by meiosis. Events occurring during meiosis creates gametes with unique combinations of gene variants and so creates genetic variability.

Sexual reproduction rearranges and reshuffles the genetic material into new combinations. This is why family members may look similar but they'll never be identical (except for identical twins).



Mitosis produces genetically identical cells. This characteristic allows the body to produce cells to heal itself when it is damaged and is also responsible for the production of the cells required for growth.

1. (a) Where does mitosis take place in animals? _____

   (b) Describe the roles of mitosis in the human body: _____

   _____

   (c) In mitosis, the daughter cells are genetically different from the parent cell. True or False? _____

2. (a) Where does meiosis take place in animals? _____

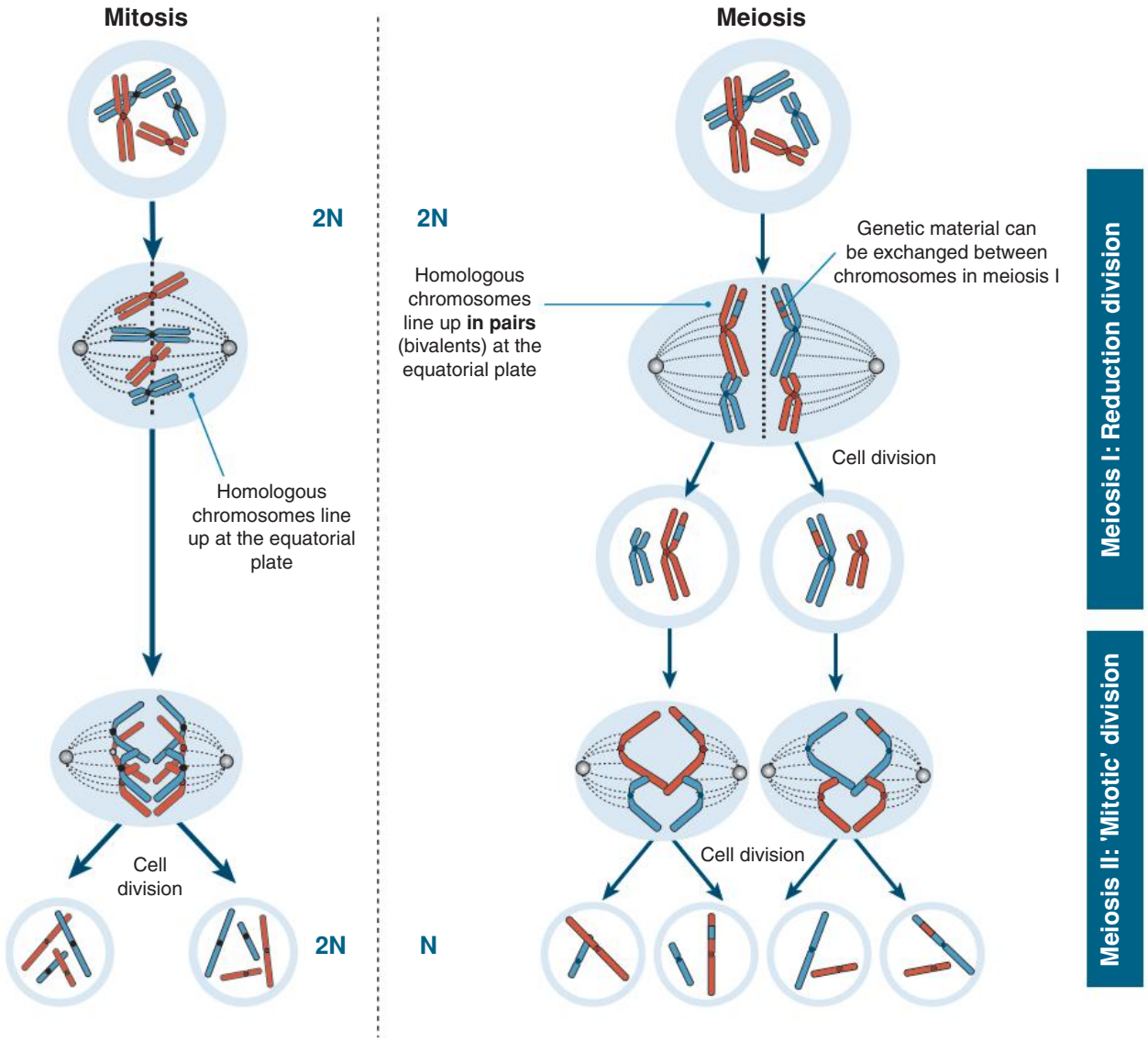
   (b) What is the purpose of meiosis? _____

   _____

   (c) In meiosis, the sex cells are genetically different to the parent cell. True or False? _____

ISBN: 978-1-98-856655-9

- **Mitosis** and **meiosis** have quite different purposes and genetic outcomes.
- Mitosis is the simpler process and produces two identical daughter cells from each parent cell. It is responsible for growth and repair in multicellular organisms and reproduction in single-celled and asexual eukaryotes.
- Meiosis involves a **reduction division** in which haploid gametes are produced for the purposes of sexual reproduction.



3. Explain how mitosis conserves chromosome number, while meiosis reduces the number from diploid to haploid:

4. Describe a fundamental difference between the first and second divisions of meiosis: _____

5. How does meiosis introduce genetic variability into gametes and offspring following gamete fusion in fertilisation?

6. If the DNA in a chromatid was affected by a mutation in a cell about to undergo meiosis, what is the probability of a daughter cell being affected by the mutation?

ISBN: 978-1-98-856655-9

38 The Eukaryotic Cell Cycle

Key Idea: The eukaryotic cell cycle can be divided into phases, although the process is continuous. Specific cellular events occur in each phase.

The life cycle of a eukaryotic cell is called the cell cycle. The cell cycle can be divided into interphase and M phase. Aspects of the cell cycle can vary enormously between cells of the same organism. For example, intestinal cells divide around twice a day, while cells in the liver divide once a year, and those in muscle tissue do not divide at all. However, if any of these tissues is damaged, cell division increases rapidly until the damage is repaired. This variety of length in the cell cycle can be explained by the existence of regulatory mechanisms that are able to slow down or speed up the cell cycle in response to changing conditions.

Interphase

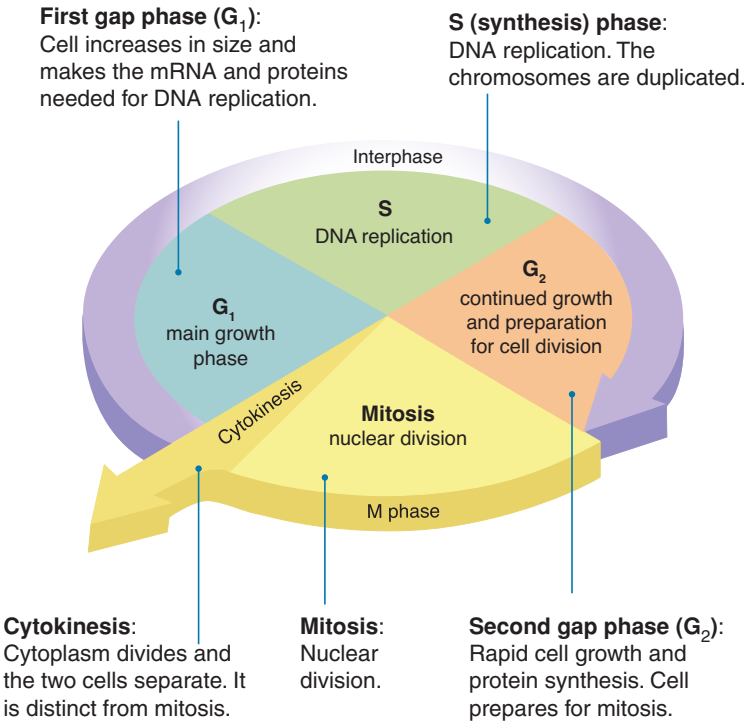
Cells spend most of their time in interphase. Interphase is divided into three stages:

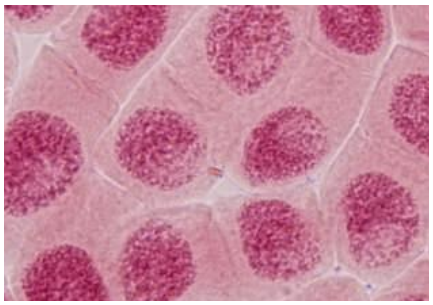
- The first gap phase (G_1)
- The S-phase (S)
- The second gap phase (G_2)

During interphase the cell increases in size, carries out its normal activities, and replicates its DNA in preparation for cell division. Interphase is not a stage in mitosis.

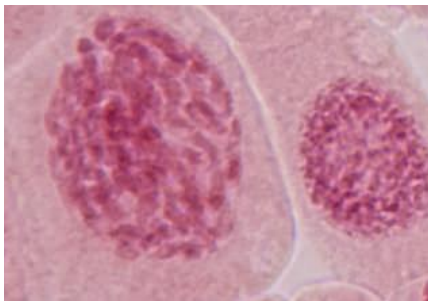
Mitosis and cytokinesis (M-phase)

Mitosis and cytokinesis occur during M-phase. During mitosis, the cell nucleus (containing the replicated DNA) divides into two equal parts. Cytokinesis occurs at the end of M-phase. During cytokinesis the cell cytoplasm divides and two new daughter cells are produced.

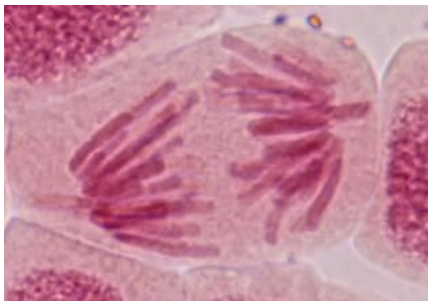




During interphase, the cell grows and acquires the materials needed to undergo mitosis. It also prepares the nuclear material for separation by replicating it.



During interphase the nuclear material is unwound. As mitosis approaches, the nuclear material begins to reorganise in readiness for nuclear division.



During mitosis the chromosomes are separated. Mitosis is a highly organised process and the cell must pass checkpoints before it proceeds to the next phase.

1. Briefly outline what occurs during the following phases of the cell cycle:

(a) Interphase: _____

_____

_____

(b) Mitosis: _____

_____

_____

(c) Cytokinesis: _____

_____

_____

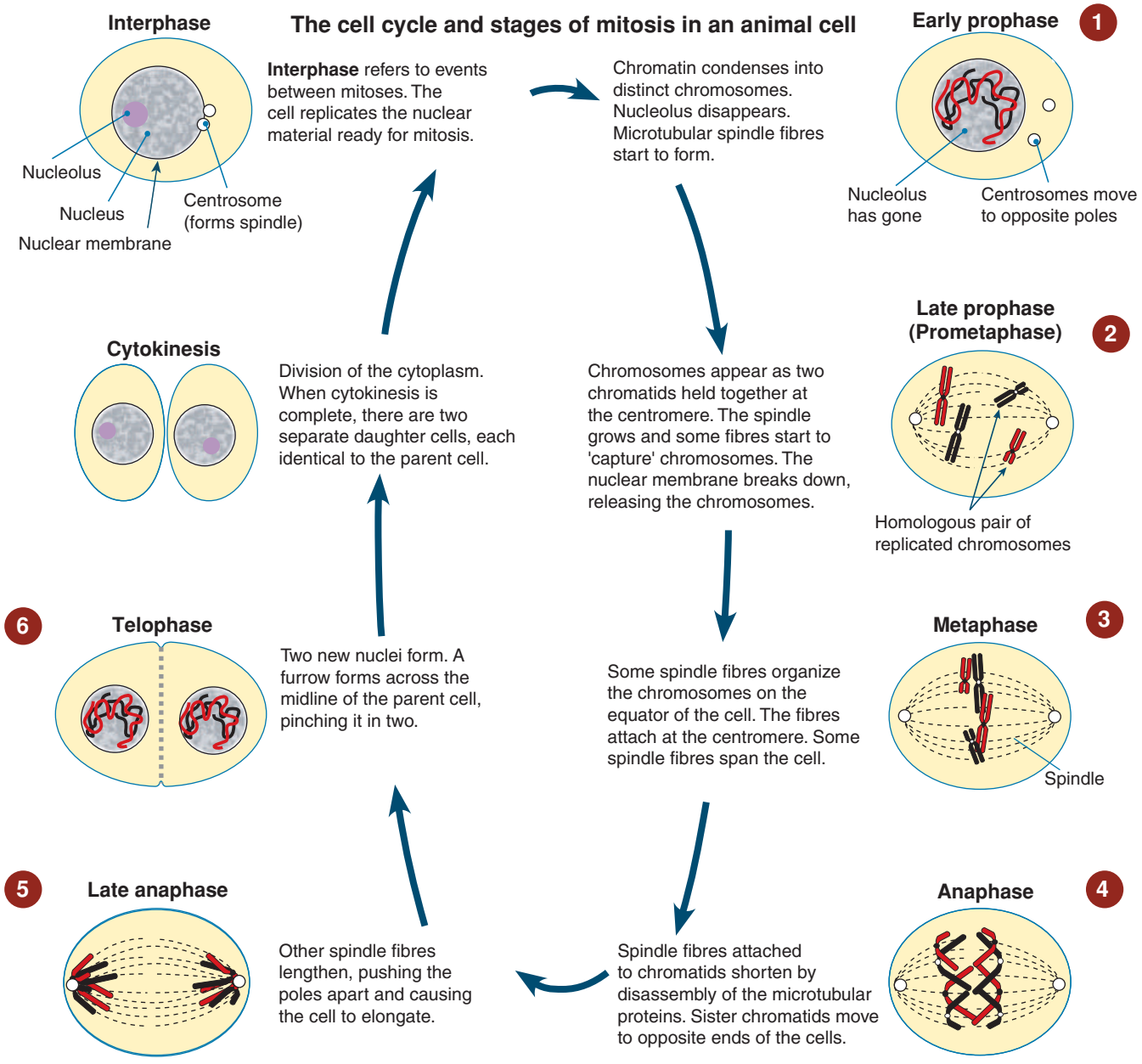
ISBN: 978-1-98-856655-9

39 Mitosis and Cytokinesis

Key Idea: Mitosis is part of the cell cycle in which an existing cell (the parent cell) divides into two (the daughter cells).

Mitosis refers to the separation (division) of the nuclear material and it precedes division of the cell. There is no change of chromosome number and the daughter cells are identical to the parent cell. Although mitosis is part of a continuous cell cycle, it is divided into stages (prophase, metaphase, anaphase, and telophase) to help distinguish the processes involved. Mitosis is one of the shortest stages of the cell cycle. Cytokinesis (the division of the newly formed cells) is part of M-phase but it is distinct from nuclear division. During cytokinesis the cell divides into two.

The cell cycle and stages of mitosis in an animal cell



1. What is the purpose of mitosis? _____

_____

_____

2. What must occur before mitosis takes place? _____

3. (a) What is the purpose of the spindle fibres? _____

_____

(b) Where do the spindle fibres originate? _____

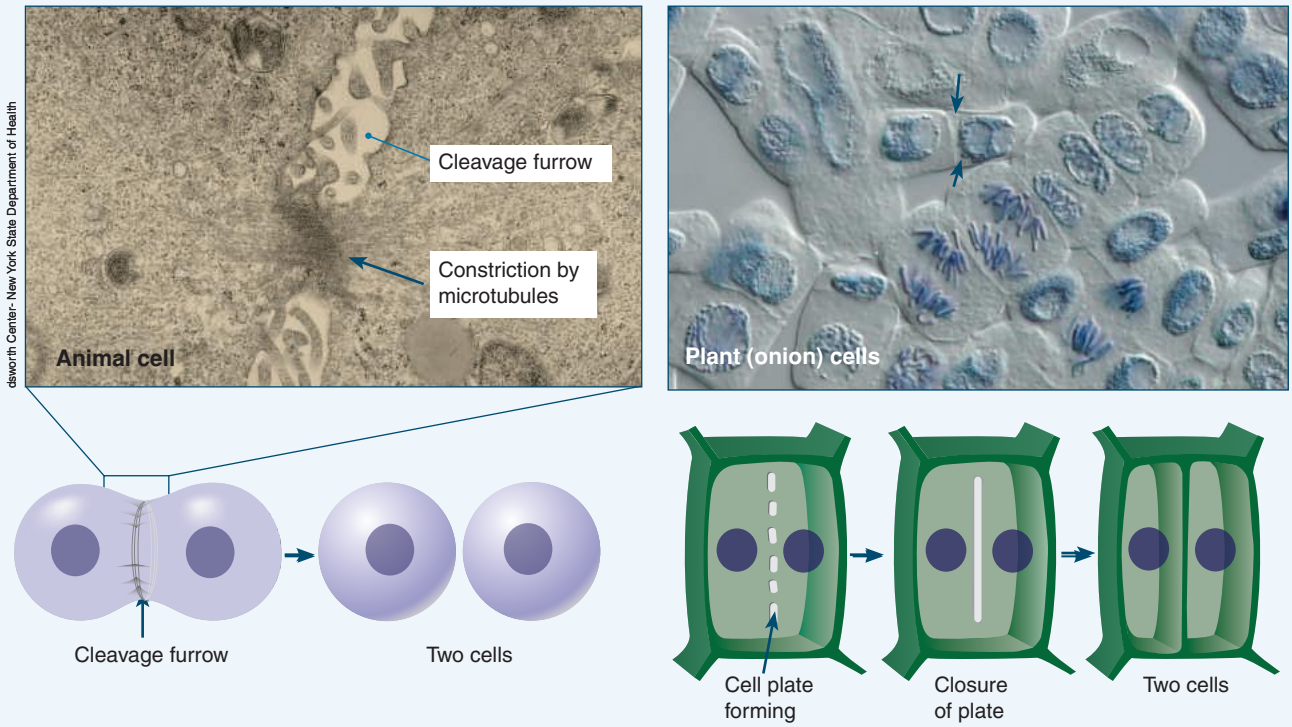
_____

ISBN: 978-1-98-856655-9

Cytokinesis (division of the cytoplasm)

Animal cells: Cytokinesis (below left) begins shortly after the sister chromatids have separated in anaphase of mitosis. A ring of microtubules assembles in the middle of the cell, next to the plasma membrane, constricting it to form a cleavage furrow. In an energy-using process, the cleavage furrow moves inwards, forming a region where the two cells will separate.

Plant cells (below right): Cytokinesis involves construction of a cell plate, a precursor of the new cell wall, in the middle of the cell. The cell wall materials are delivered by vesicles derived from the Golgi. The vesicles join together to become the plasma membranes of the new cell surfaces.



Cytokinesis in an animal cell **Cytokinesis in a plant cell**

4. Summarise what happens in each of the following phases:

   (a) Prophase: _____

   _____

   (b) Metaphase: _____

   _____

   (c) Anaphase: _____

   _____

   (d) Telophase: _____

   _____

   _____

5. (a) What is the purpose of cytokinesis? _____

   _____

   (b) Describe the differences between cytokinesis in an animal cell and a plant cell: _____

   _____

   _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

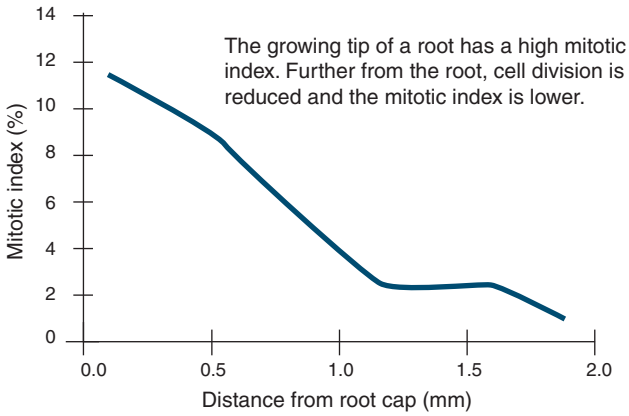
40 Recognising Stages in Mitosis

Key Idea: The stages of mitosis can be recognised by the organisation of the cell and chromosomes.
Although mitosis is a continuous process it is divided into four stages (prophase, metaphase, anaphase, and telophase) to more easily describe the processes occurring during its progression.

The mitotic index

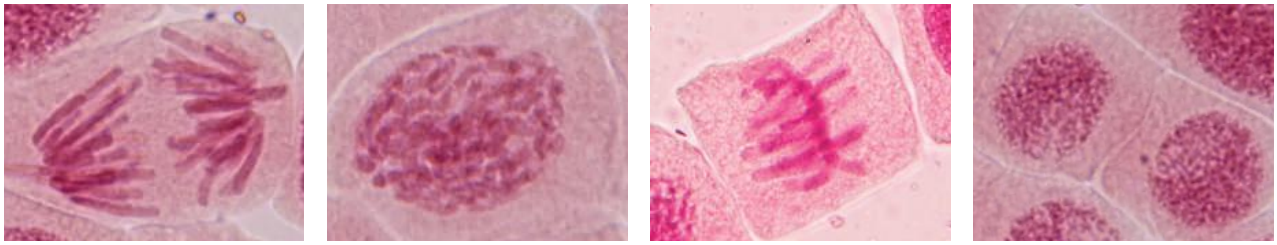
The mitotic index measures the ratio of cells in mitosis to the number of cells counted. It is a measure of cell proliferation and can be used to diagnose cancer because cancerous cells divide very quickly. In areas of high cell growth the mitotic index is high, such as in plant apical meristems or the growing tips of plant roots. The mitotic index can be calculated using the formula below:

$$\text{Mitotic index} = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells}}$$



The growing tip of a root has a high mitotic index. Further from the root, cell division is reduced and the mitotic index is lower.

1. Use the information in the previous activity to identify which stage of mitosis is shown in each of the photographs below:



(a) _____ (b) _____ (c) _____ (d) _____

2. (a) The light micrograph (right) shows a section of cells in an onion root tip. These cells have a cell cycle of approximately 24 hours. The cells can be seen to be in various stages of the cell cycle. By counting the number of cells in the various stages it is possible to calculate how long the cell spends in each stage of the cycle. Count and record the number of cells in the image that are in mitosis and those that are in interphase. Cells in cytokinesis can be recorded as in interphase. Estimate the amount of time a cell spends in each phase.

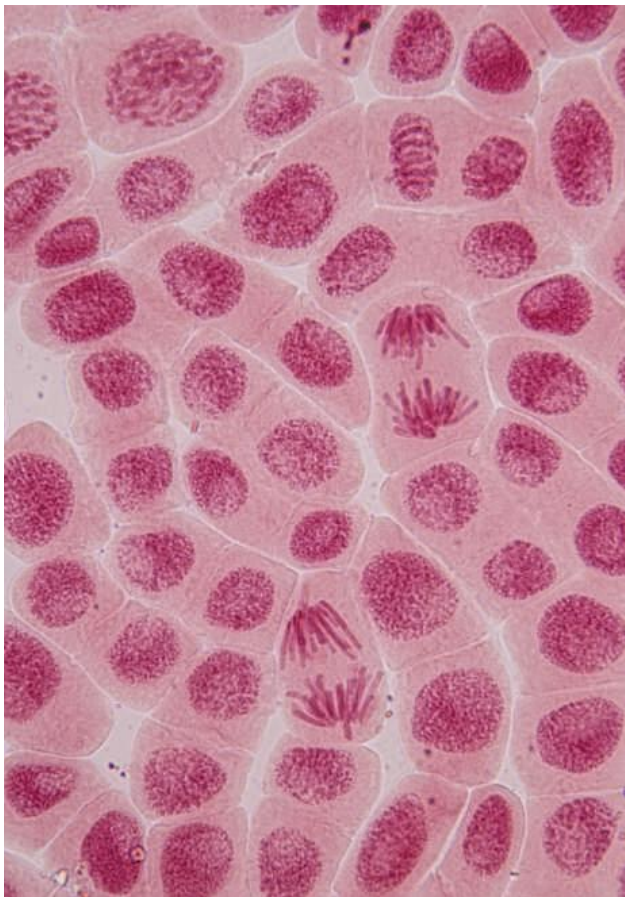
Stage	No. of cells	% of total cells	Estimated time in stage
Interphase			
Mitosis			
Total		*100*	

(b) Use your counts from 2(a) to calculate the mitotic index for this section of cells.

_____

_____

Onion root tip cells



3. What would you expect to happen to the mitotic index of a population of cells that loses the ability to divide as they mature?

_____

_____

ISBN: 978-1-98-856655-9

41 Modelling Mitosis

Key Idea: Using pipe cleaners to model the stages of mitosis will help you to visualise and understand the process.

Modelling clay or pipe cleaners and string can be used to model stages of mitosis in an animal cell. For simplicity, it is easiest to start with 4 chromosomes (2N = 4).

Investigation 2.1 Modelling mitosis

See appendix for equipment list.

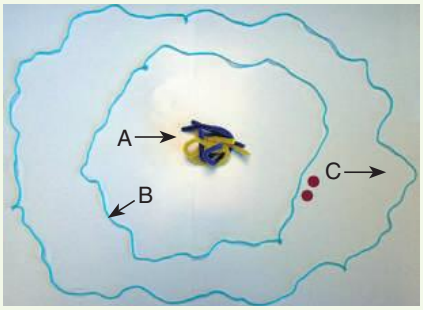
You can work in pairs for this activity if you wish.

1. Use the information on the previous pages to model mitosis in an animal cell using pipe cleaners and string. Work in pairs and use four chromosomes for simplicity (2N = 4). Photograph or film each stage.
2. Photo 1 (below) can be used as a starting point for your model. It represents a cell in interphase before mitosis begins. The circular structures are the replicated centrosomes.
3. Before you start, identify the structures A–C in photo

   A: _____ B: _____ C: _____

4. Remember to label your photos as you place them on the page.

Photo 1



ISBN: 978-1-98-856655-9

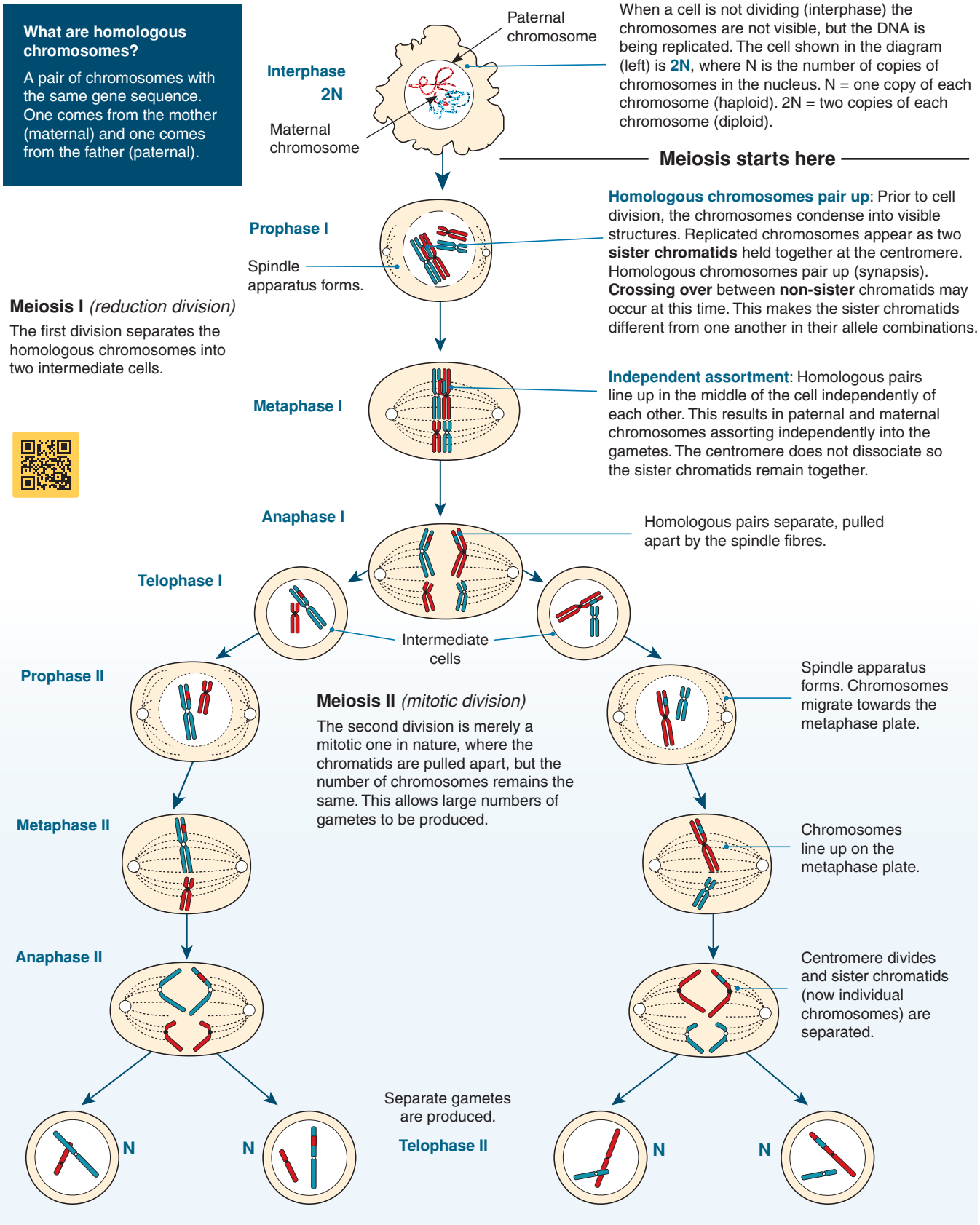
42 Meiosis

Key Idea: Meiosis is a special type of cell division. It produces sex cells (gametes) for the purpose of sexual reproduction.

Meiosis involves a single chromosomal duplication followed by two successive nuclear divisions, and results in halving of the diploid chromosome number. Meiosis occurs in the sex organs of animals and the sporangia of plants. If genetic mistakes (**gene** and **chromosome mutations**) occur here, they will be passed on to the offspring, i.e. they will be inherited. Meiosis creates genetic variation in the sex cells through crossing over and independent assortment. Crossing over refers to the mutual exchange of pieces of chromosome, and their genes, between homologous chromosomes. In independent assortment, homologous chromosomes are randomly distributed to the gametes.

What are homologous chromosomes?

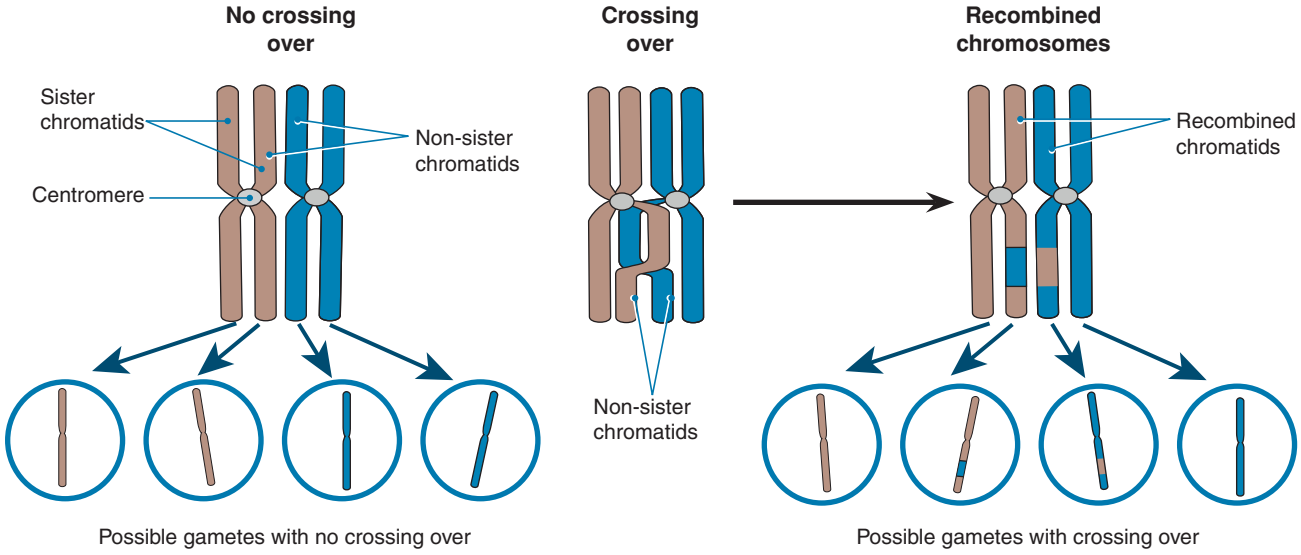
A pair of chromosomes with the same gene sequence. One comes from the mother (maternal) and one comes from the father (paternal).



ISBN: 978-1-98-856655-9

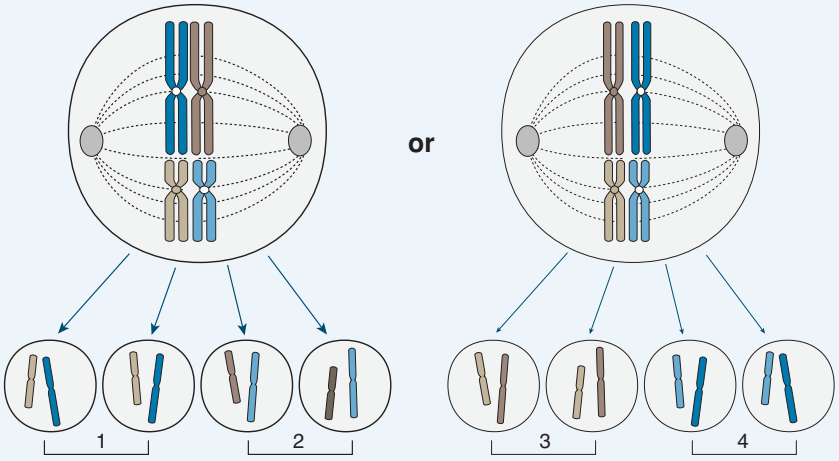
Crossing over and recombination

Chromosomes replicate during interphase, before meiosis, to produce replicated chromosomes, with sister chromatids held together at the centromere (see below). When the replicated chromosomes are paired during the first stage of meiosis, non-sister chromatids may become entangled and segments may be exchanged in a process called **crossing over**. Crossing over results in the **recombination** of alleles (variations of the same gene) producing greater variation in the offspring than would otherwise occur.



Independent assortment

Independent assortment is the random alignment and distribution of chromosomes during meiosis. Independent assortment is an important mechanism for producing variation in gametes. During the first stage of meiosis, replicated homologous chromosomes pair up along the middle of the cell. The way the chromosomes pair up is random. For the homologous chromosomes (right), there are two possible ways in which they can line up resulting in four different combinations in the gametes. The intermediate steps of meiosis have been left out for simplicity.



1. Describe the behaviour of the chromosomes in the first, and then the second division in meiosis: _____

2. How does independent assortment increase the variation in gametes? _____

3. (a) What is crossing over? _____

   (b) How does crossing over increase the variation in the gametes, and hence the offspring? _____

ISBN: 978-1-98-856655-9

43 Modelling Meiosis

Key Idea: We can simulate crossing over, gamete production, and the inheritance of alleles during meiosis using ice-block sticks to represent chromosomes.

This practical activity simulates the production of gametes (sperm and eggs) by meiosis and shows you how crossing over increases genetic variability. This is demonstrated by studying how two of your own alleles are inherited by the 'child' produced at the completion of the activity. Completing this activity will help you to visualise and understand some of the important aspects of events in meiosis.

Investigation 2.2 Modelling meiosis using ice-block sticks

See appendix for equipment list.

To study the effect of crossing over on genetic variation, you will work in pairs to simulate the inheritance of two of your own traits: ability to tongue roll and handedness. This activity will take 25–45 minutes.

1. Record your phenotype and genotype for each trait in the table (right). If you have a dominant trait, you will not know if you are heterozygous or homozygous for that trait, so you can choose either genotype.
2. Before you start the simulation, partner up with a classmate. Your gametes will combine with theirs (fertilisation) at the end of the activity to produce a 'child'. Decide who will be female, and who will be male. You will need to work with this person again at step 7.
3. Collect four ice-block sticks. These represent four chromosomes. Colour two sticks blue or mark them with a P. These are the paternal chromosomes. The plain sticks are the maternal chromosomes. Write your initials on each of the four sticks. Label each chromosome with its number. Label four sticky dots with the alleles to describe your phenotype and stick each onto the appropriate chromosome. In the example shown, the person is heterozygous for tongue rolling so sticky dots with alleles T and t are placed on chromosome 10. The person is also left handed, so alleles r and r are placed on chromosome 2 (right).
4. Randomly drop the chromosomes onto a table. This represents a cell in either the testes or ovaries.

   Duplicate your chromosomes by adding four more identical ice-block sticks to the table (right).
   What are you simulating with this action?

   _____

5. Simulate the first stage of meiosis by lining the duplicated chromosome pair with their homologous pair (below). For each chromosome number, you will have four sticks touching side-by-side (A, below).

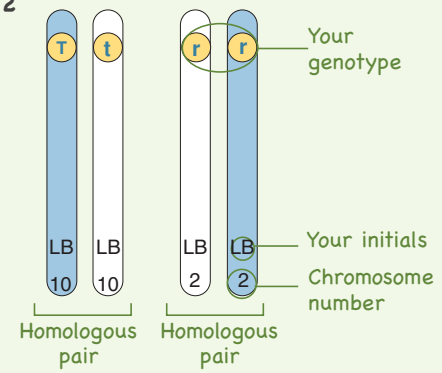
   At this stage crossing over occurs. Simulate this by swapping sticky dots from adjoining homologues (B, below).

Chromosome number	Phenotype	Genotype
10	Tongue roller	TT, Tt
10	Non-tongue roller	tt
2	Right handed	RR, Rr
2	Left handed	rr

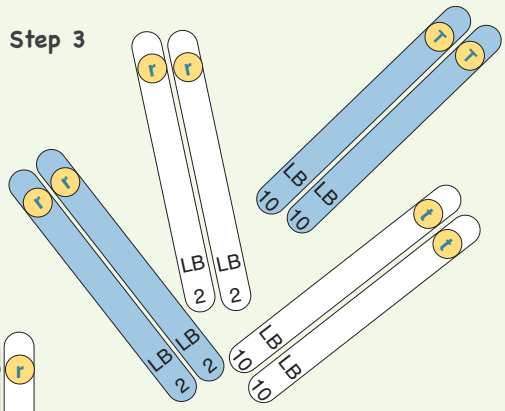
Step 1

Trait	Phenotype	Genotype
Handedness		
Tongue rolling		

Step 2

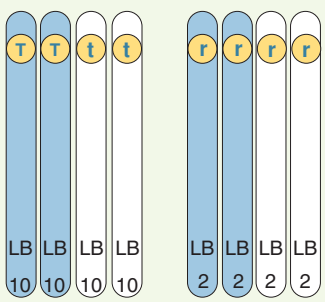


Step 3

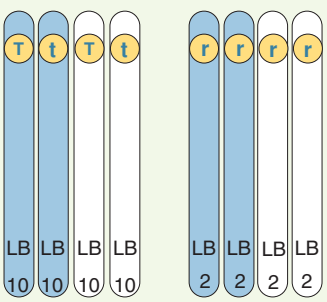


Step 4

(A)



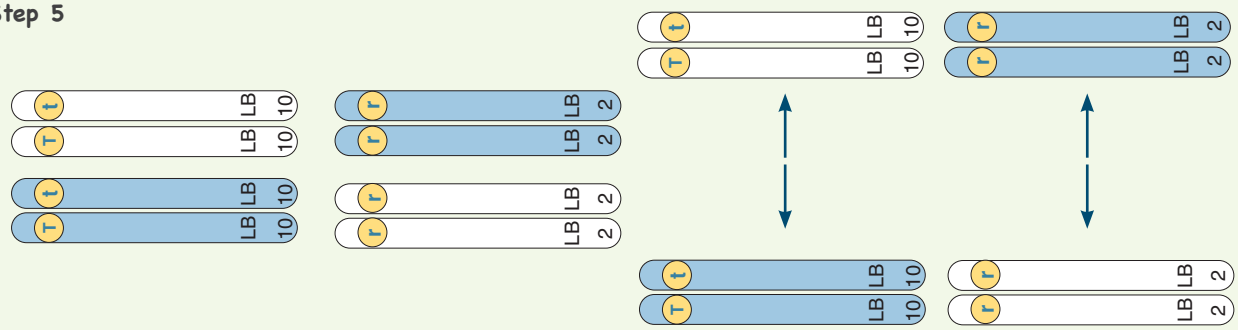
(B)



ISBN: 978-1-98-856655-9

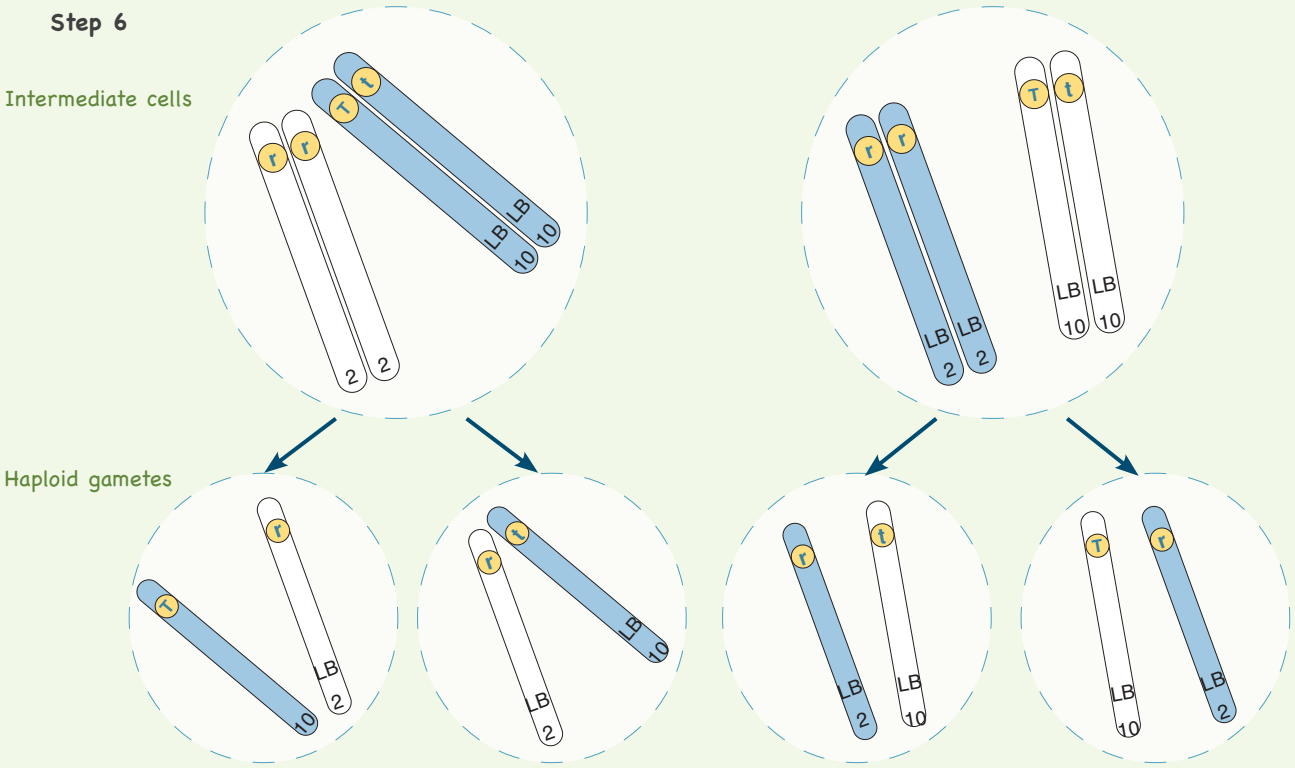
6. Randomly align the homologous chromosome pairs to simulate alignment across the cell's centre (equator) (as occurs in the next phase of meiosis). Simulate the separation of the chromosome pairs.
For each group of four sticks, two are pulled to each pole (end) of the cell.

Step 5



7. Two intermediate cells are formed. If you have completed step 5 correctly, each intermediate cell will be haploid (half the diploid chromosome number shown in step 3) with a mixture of maternal and paternal chromosomes. This is the end of the first division of meiosis. Your cells now need to divide for a second time. Repeat steps 4 and 5 but this time there is no crossing over and you are now separating replicated chromosomes, not homologues. At the end of this process each intermediate cell will have produced two haploid gametes. Each will have a maternal chromosome (white) and a paternal chromosome (blue) (below).

Step 6



8. Pair up with the partner you chose at the beginning of the exercise to carry out fertilisation. Randomly select one sperm and one egg cell. The unsuccessful gametes can be removed from the table. Combine the chromosomes of the successful gametes. You have created a child! Fill in the following chart to describe your child's genotype and phenotype for tongue rolling and handedness.

Trait	Phenotype	Genotype
Handedness		
Tongue rolling		

ISBN: 978-1-98-856655-9

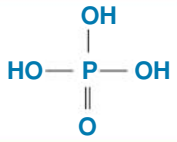
44 Nucleotides

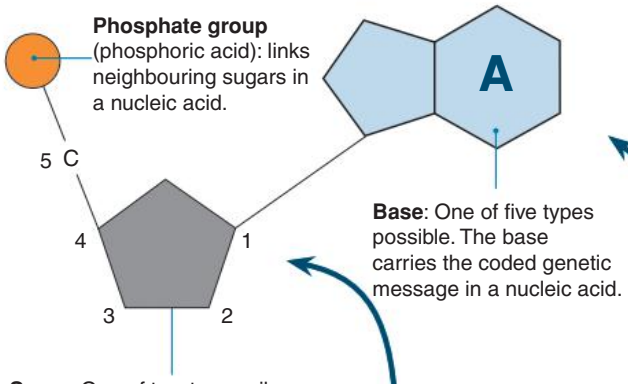
Key Idea: Nucleotides make up nucleic acids. A nucleotide is made up of a base, a sugar, and a phosphate group.

Nucleotides are the building blocks of DNA and RNA, the nucleic acids that are involved in the transmission of inherited information. Nucleotide derivatives, such as ATP and GTP, are involved in energy transfers in cells. A nucleotide has three components: a base, a sugar, and a phosphate group. Nucleotides may contain one of five bases. The combination of bases in the nucleotides making up DNA or RNA stores the information controlling the cell's activity. The bases in DNA are the same as RNA except that thymine (T) in DNA is replaced with uracil (U) in RNA.

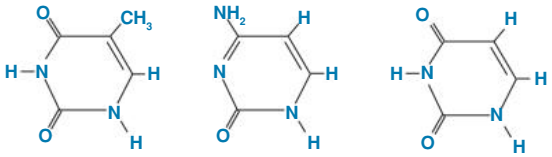
Phosphate

Phosphate groups are represented by orange circles. With the pentose sugar they form the 'backbone' of the DNA or RNA molecule.



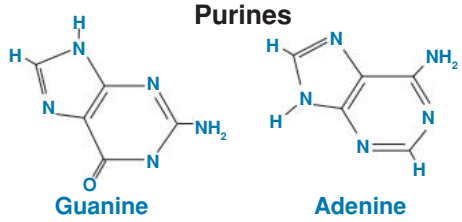


Pyrimidines



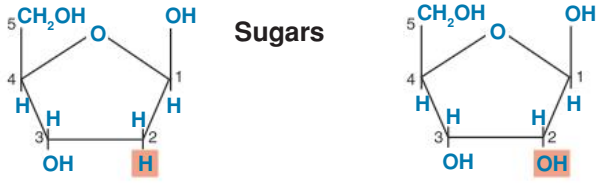
Pyrimidines are single ringed bases. DNA contains the pyrimidines cytosine (C) and thymine (T). RNA contains the pyrimidines cytosine and uracil (U).

Purines



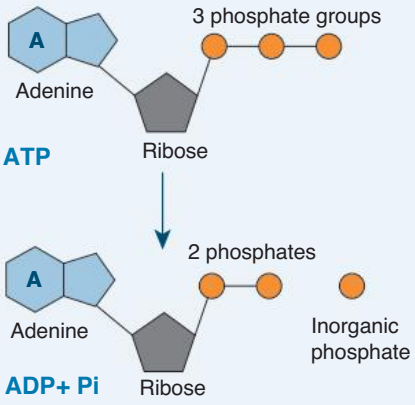
Purines are double ringed bases. Both DNA and RNA contain the purines adenine (A) and guanine (G).

Sugars



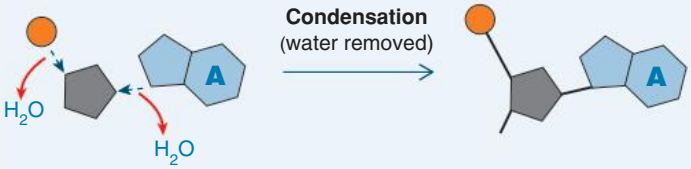
Nucleotides contain one of two different sorts of sugars. **Deoxyribose** sugar is found in DNA. **Ribose** sugar is found in RNA.

Nucleotide derivatives



ATP is a nucleotide derivative that provides chemical energy for metabolism. It consists of an adenine linked to a ribose sugar and 3 phosphate groups. Energy is made available when a phosphate group is transferred to a target molecule. Other nucleoside triphosphates (NTPs) have similar roles.

Nucleotide formation



In formation of a nucleotide, a phosphoric acid and a base are chemically bonded to a sugar molecule by **condensation** reactions in which water is given off. The reverse reaction is **hydrolysis**.

1. List the nucleotide bases present:

   (a) In DNA: _____

   (b) In RNA: _____

2. Name the sugar present: (a) In DNA: _____ (b) In RNA: _____

3. How can simple nucleotide units combine to store genetic information? _____

_____

_____

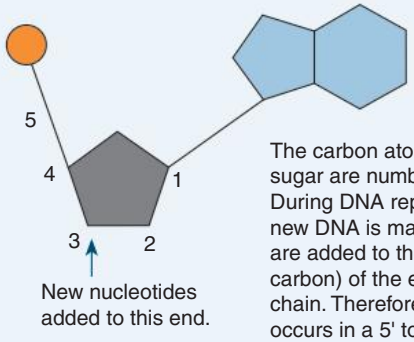
ISBN: 978-1-98-856655-9

45 Nucleic Acids

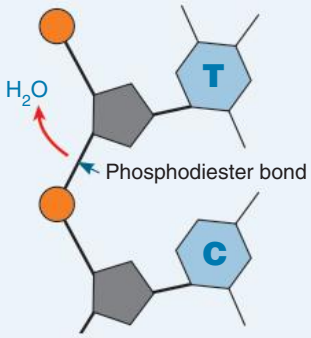
Key Idea: Nucleic acids are macromolecules made up of long chains of nucleotides, which store and transmit genetic information. DNA and RNA are nucleic acids.

DNA and RNA are nucleic acids involved in the transmission of inherited information. Nucleic acids have the capacity to store the information that controls cellular activity. The central nucleic acid is called **deoxyribonucleic acid** (DNA). **Ribonucleic acids** (RNA) are involved in the 'reading' of the DNA information. All nucleic acids are made up of nucleotides linked together to form chains or strands. The strands vary in the sequence of the bases found on each nucleotide. It is this sequence that provides the 'genetic instructions' for the cell.

Nucleotides are joined by condensation polymerisation

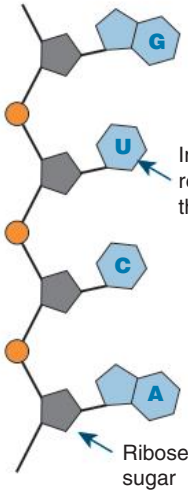


The carbon atoms on the pentose sugar are numbered one to five. During DNA replication (when new DNA is made) nucleotides are added to the 3' end (the third carbon) of the existing nucleotide chain. Therefore, DNA replication occurs in a 5' to 3' direction.



A **condensation** reaction (left) joins two molecules together with the loss of a water molecule. In the formation of nucleic acids, nucleotides are joined together into polymers through a condensation reaction between the phosphate group of one nucleotide and the sugar of another. Water is released and a **phosphodiester bond** is formed. Because of the way they are formed, nucleic acids are called **condensation polymers**.

RNA molecule

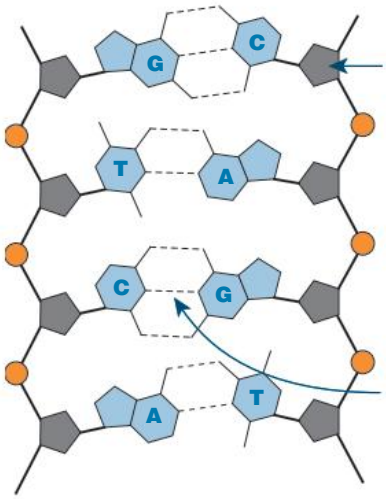


In RNA, uracil replaces thymine in the code.

Ribose sugar

Ribonucleic acid (RNA) is made up of a single strand of nucleotides linked together. Although it is single stranded, it is often found folded back on itself, with complementary bases joined by hydrogen bonds.

DNA molecule

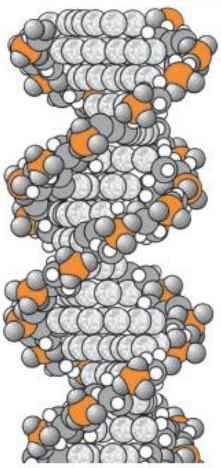


Deoxyribose sugar

Hydrogen bonds hold the two strands together.

Only certain bases can pair.

Symbolic representation



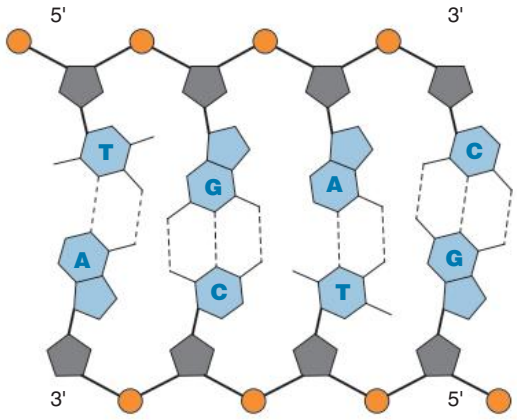
Space filling model

Deoxyribonucleic acid (DNA) is made up of a double strand of nucleotides linked together. It is shown unwound in the symbolic representation (above left). The DNA molecule takes on a double helix shape as shown in the space filling model above right.

Double-stranded DNA

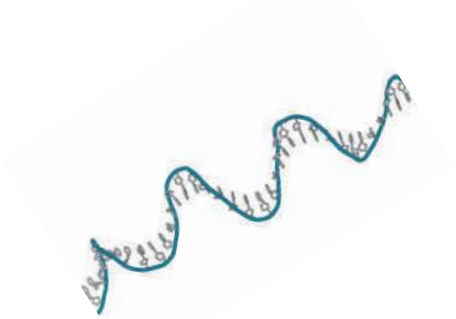
The double-helix structure of DNA is like a ladder twisted into a corkscrew shape around its longitudinal axis. It is 'unwound' here to show the relationships between the bases.

- The DNA backbone is made up of alternating phosphate and sugar molecules, giving the DNA molecule an asymmetrical structure.
- The asymmetrical structure gives a DNA strand **direction**. Each strand runs in the opposite direction to the other.
- The ends of a DNA strand are labelled the 5' (five prime) and 3' (three prime) ends. The **5'** end has a terminal phosphate group (off carbon 5), the **3'** end has a terminal hydroxyl group (off carbon 3).
- The way the pairs of bases come together to form hydrogen bonds is determined by the number of bonds they can form and the configuration of the bases.

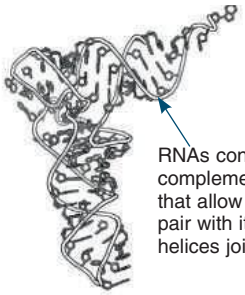


ISBN: 978-1-98-856655-9

RNAs are involved in decoding the genetic information in DNA, as messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). RNA is also involved in modifying mRNA after transcription and in regulating translation.

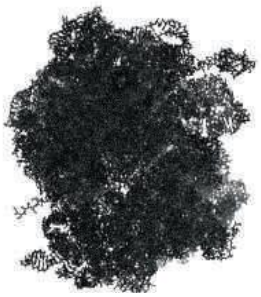


Messenger RNA (above) is transcribed (written) from DNA. It carries a copy of the genetic instructions from the DNA to ribosomes in the cytoplasm, where it is translated into a polypeptide chain.



RNAs contain self-complementary sequences that allow parts of the RNA to pair with itself to form short helices joined by H bonds.

Transfer RNA (above) carries amino acids to the growing polypeptide chain. One end of the tRNA carries the genetic code in a three-nucleotide sequence called the **anticodon**. The amino acid links to the 3' end of the tRNA.



Ribosomal RNA (above) forms ribosomes from two separate ribosomal components (the large and small subunits) and assembles amino acids into a polypeptide chain.

1. (a) In double-stranded DNA, bases in one strand always pair up with specific bases in the complementary strand. This is called the **base-pairing rule**. Use the previous diagrams to describe the base-pairing rule in double-stranded DNA:

   _____

   _____

   (b) How is the base-pairing rule for RNA different? _____

   (c) What is the purpose of the hydrogen bonds in double-stranded DNA? _____

   _____

2. Briefly describe the roles of RNA: _____

   _____

   _____

3. (a) If you wanted to use a radioactive or fluorescent tag to label only the RNA in a cell and not the DNA, what molecule(s) would you label?

   _____

   (b) If you wanted to use a radioactive or fluorescent tag to label only the DNA in a cell and not the RNA, what molecule(s) would you label?

   _____

4. (a) Why do the DNA strands have an asymmetrical structure? _____

   _____

   _____

   (b) What are the differences between the 5' and 3' ends of a DNA strand? _____

   _____

5. Complete the following table summarising the differences between DNA and RNA molecules:

	DNA	RNA
Sugar present		
Bases present		
Number of strands		
Relative length		

ISBN: 978-1-98-856655-9

46 What Does DNA Look Like?

Key Idea: Strands of DNA can be extracted from any cells, but those with large amounts of DNA and large chromosomes produce the best results.

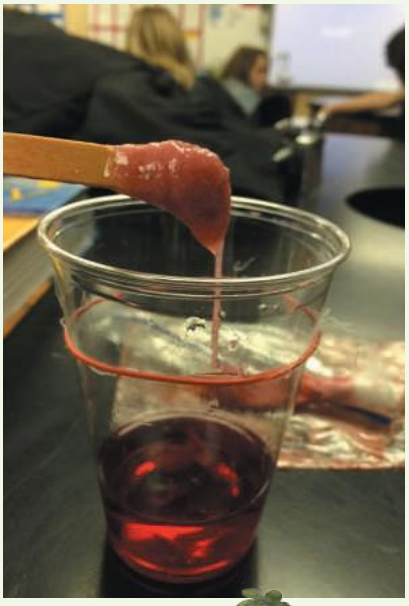
In a lab, scientists usually use extraction kits to separate DNA from cells. These will contain all the parts needed to accurately remove the DNA, but in general they are all based on the same technique. This includes breaking open the cells, precipitating the DNA, and removing contaminants. In a classroom, DNA is easily extracted by precipitating it out of solution using ice cold ethanol. It is good to use strawberries for this method because they are octaploid (have 8 sets of chromosomes) and their colour makes it easy to see the precipitating DNA. However, other fruits and vegetables such as kiwifruit, bananas, and broccoli can also be used.

Investigation 2.3 Extracting DNA

See appendix for equipment list.

Work in pairs for this activity.

1. Take 5-6 strawberries and place them in a large zip-lock bag. Squash the strawberries into a smooth paste. This mechanically breaks up the cells, but does not release the DNA.
2. To release the DNA add 100 mL of water, 5 mL of detergent, and a pinch of salt to the paste. Reseal the bag and mix the contents by squashing and crumpling the bag. The detergent breaks down the cellular membranes and deactivates DNases, which would chop up the DNA. The salt helps to remove the proteins bound to the DNA and keeps them in solution. Positive ions in the salt also neutralise the negative charge of the DNA.
3. Place a piece of filter paper in a funnel and position the funnel so the excess fluid can drain into a beaker. Pour the contents of the bag into the filter funnel and allow it to drain. It should produce a clear reddish solution (right).
4. Gently add the ethanol on top of the strawberry solution by placing a clean glass rod into the beaker and carefully pouring the ethanol down the rod. Add ethanol until there are equal volumes of strawberry solution and ethanol.
5. Ethanol removes the water from around the DNA so it precipitates where the ethanol and the solution meet, forming whitish glue-like strands. Low temperatures speed up the precipitation and limit DNase activity.
6. The DNA strands can be centrifuged with ethanol to isolate the DNA as a pellet.



Yaminchhipa10 CC 4.0

1. In the extraction and isolation of DNA:

   (a) Why is it necessary to disrupt the cellular membranes? _____

   (b) Why does the DNA precipitate out in ethanol? _____

   (c) For a DNA extraction, why is it helpful that strawberries are octaploid? _____

   (d) Why is salt added? _____

   _____

   (e) What is the purpose of the filter funnel? _____

   _____

2. In a DNA extraction, student A obtained DNA in long threads, whereas student B obtained DNA that appeared fluffy. Account for the differences in these two results, and suggest what student B might have done incorrectly:

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

47 Creating a DNA Model

Key Idea: Nucleotides pair according to the base pairing rule. There are ten base pairs per turn of the DNA double helix.

DNA is made up of structures called nucleotides. Two primary factors control the way in which these nucleotide building blocks are linked together: 1) the available space within the DNA double helix and 2) the hydrogen-bonding capability of the bases. These factors cause the nucleotides to join together in a predictable way, referred to as the **base pairing rule**. The strands of the DNA are antiparallel (they run in opposite directions) and there are 10 base pairs per 360° turn of the helix. The activity below will guide you through constructing a three dimensional model of DNA.

DID YOU KNOW?

Chargaff's rules

Before Watson and Crick described the structure of DNA, an Austrian chemist called Chargaff analysed the base composition of DNA from a number of organisms. He found that the base composition varies between species but that within a species the percentage of A and T bases are equal and the percentage of G and C bases are equal. Validation of Chargaff's rules was the basis of Watson and Crick's base pairs in the DNA double helix model.

DNA base pairing rule

Adenine	always pairs with	**Thymine**	A ⟷ T
Thymine	always pairs with	**Adenine**	T ⟷ A
Cytosine	always pairs with	**Guanine**	C ⟷ G
Guanine	always pairs with	**Cytosine**	G ⟷ C

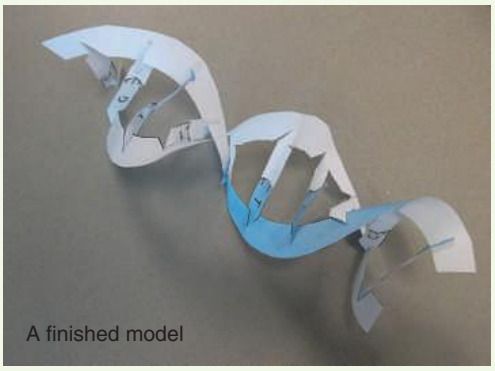
Investigation 2.4 Creating a model of a DNA molecule

See appendix for equipment list.

Work in pairs for this activity.

1. Cut out the opposite page. Cut out the template strand. Dark black lines should be cut. Make a slight fold on the red dashed lines so that the grey surfaces are facing (a valley fold). Do not cut around the grey representations of hydrogen bonds on each base. These are to show you where you will join your bases.
2. Cut out the complementary strand. The first base (G) is in position as a guide. Fold on the red dashed line so that the blue surfaces are facing each other.
3. Fill in the table (right) to help you place the remaining bases in the correct order (a)–(i) on the complementary strand:
4. Cut out the bases and slot them into the slots on the complementary strand using the order in the table above. Use short lengths of tape to fix them in position. Make sure the blue surfaces are facing and the base is in the same orientation as the guide (G).
5. Line up the first base pairs (C and G) and stick them together with tape. The tape takes the place of the hydrogen bonds holding the strands together. Note that the bases are facing in opposite directions.
6. Continue sticking base pairs together, working your way around the helix, to complete the DNA molecule.
7. Together, or in groups, search online for at least three different representations of a DNA molecule. Evaluate your model against these representations. How are they similar? How are they different? If you wish, attach pictures of the DNA representations you selected to this page.

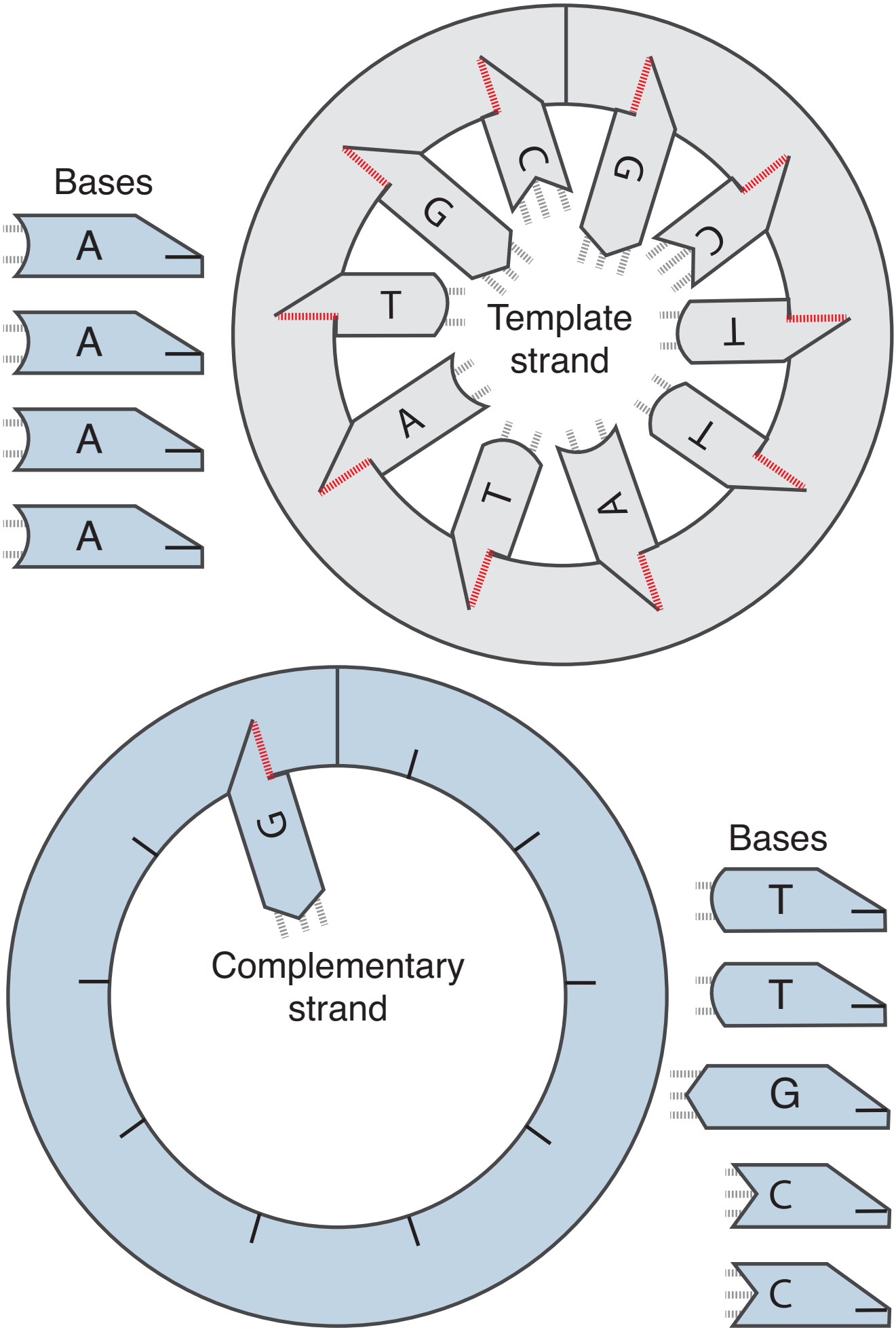
Template strand	Complementary strand
Cytosine (C)	Guanine (G)
Guanine (G)	(a)
Thymine (T)	(b)
Adenine (A)	(c)
Thymine (T)	(d)
Adenine (A)	(e)
Thymine (T)	(f)
Thymine (T)	(g)
Cytosine (C)	(h)
Guanine (G)	(i)



A finished model

1. Describe your model in terms of the other representations you looked at. What are its strengths and deficiencies?

ISBN: 978-1-98-856655-9



ISBN: 978-1-98-856655-9

The page has been deliberately left blank

ISBN: 978-1-98-856655-9

48 The Evidence for DNA Structure

Key Idea: Many scientists contributed to the discovery of DNA's structure. Once the structure of DNA was known, it immediately suggested a mechanism for its replication.

DNA is easily extracted and isolated from cells. This was first done in 1869, but it took the work of many scientists working in different areas many years to determine DNA's structure. In particular, four scientists, Watson, Crick, Franklin, and Wilkins are recognised as having made significant contributions in determining the structure of DNA. Once the structure of DNA was known, scientists could determine how it was replicated.

Discovering the structure of DNA ... a story of collaboration and friction

Although Watson and Crick are often credited with discovering DNA's structure,the contributions of many scientists were important. This includes not only the contributions from scientists at the time, but also from earlier researchers whose findings contributed to the body of existing knowledge.

Personal conflicts and internal politics probably prevented DNA's structure being determined earlier. Professional friction between Rosalind Franklin and Maurice Wilkins meant that they worked independently of each other. Watson and Crick analysed some of Rosalind Franklin's results, notably 'photo 51', without her knowledge or consent and Watson himself recalls that he tended to dismiss her. Photo 51 was crucial to Watson and Crick's model because it showed that DNA was a double helix. Only later did he acknowledge her considerable contribution.

Franklin was conservative by nature and opposed to prematurely building theoretical models until there was enough data to guide the model building. However, when she saw Watson and Crick's model, she readily accepted it. Despite her contribution, Franklin did not receive the Nobel prize, which cannot be awarded posthumously.

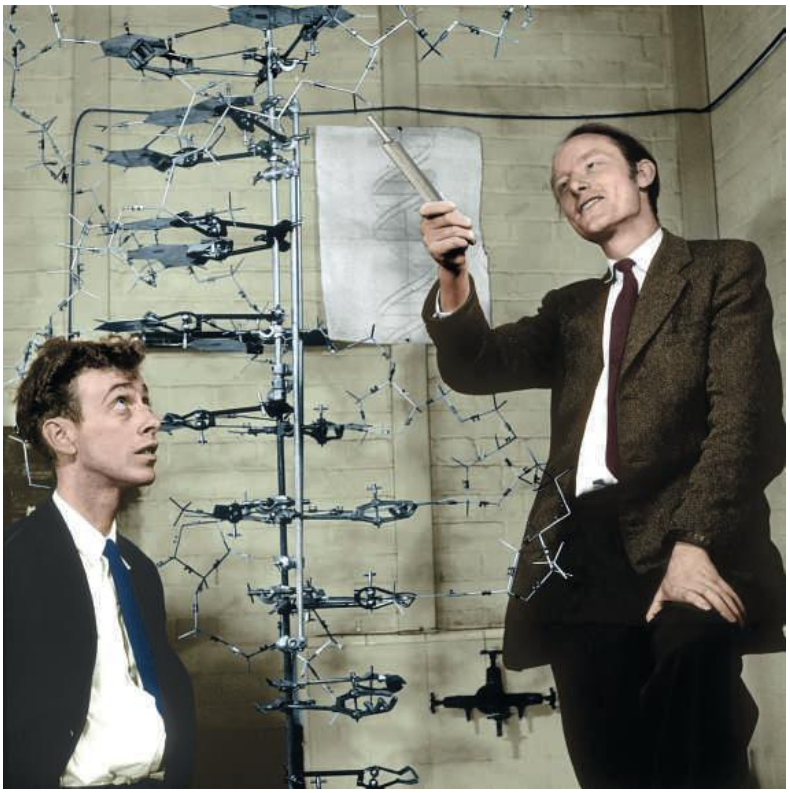


IMAGE: A. Barrington-Brown, © Gonville and Caius College, Cambridge / Coloured by Science Photo Library

James Watson (left) and Francis Crick (right) in 1953 with their DNA model.

Late 1940s
Linus Pauling determined by x-ray crystallography that proteins have a helical structure.

1951
Rosalind Franklin studied the structure of DNA using x-ray crystallography at King's College. Maurice Wilkins, also at King's College, was also using x-ray crystallography to study DNA. Wilkins and Franklin did not get on well.

30 January 1953
Wilkins shows Watson and Crick the 'photo 51' without Franklin's approval or knowledge. It provided the structural information they needed to finalise their model, completed on 7 March 1953.

16 April 1958
Franklin dies at age 37 of ovarian cancer. She was never nominated for a Nobel Prize.

1949
Chargaff's rules are announced: DNA contains equal proportions of bases A and T and G and C.

1951
James Watson and Francis Crick build their first DNA model - a three stranded helix, with bases to the outside and phosphate groups to the inside. Franklin points out that their model is incorrect and is not consistent with the data.

May 1952
Franklin produces 'photo 51', showing DNA is a helix. She was working on a less hydrated form of DNA and did not return to the photo again until 1953.

1962
Watson, Crick, and Wilkins win the Nobel Prize in Physiology or Medicine. Franklin was not acknowledged.

1. As a class, work together to produce a timeline of the events leading to the discovery of DNA's structure. In small groups, choose one event or researcher and explain its/their significance. You can use the information presented in this activity, as well as any information provided on **BIOZONE's Resource Hub**. Take a photograph of your completed timeline and attach it to this page. How much was the discovery of DNA's structure a collaboration between scientists working in related fields? Were Watson and Crick wrong to use information they did not have permission to use? How much did their less cautious approach accelerate the determination of DNA's structure? Attach any extra notes or comments to this page.

ISBN: 978-1-98-856655-9

49 How Does DNA Replicate?

Key Idea: Semi-conservative DNA replication produces two identical copies of DNA, each containing half original material and half new material.

Before a cell can divide, it must double its DNA. It does this by a process called DNA replication. This process ensures that each resulting cell receives a complete set of genetic instructions from the parent cell. After the DNA has replicated, each chromosome is made up of two chromatids joined at the centromere. The two chromatids will become separated during cell division to form separate chromosomes. During DNA replication, nucleotides are added at the replication fork. Enzymes are responsible for all of the key events.

Step 1
Unwinding the DNA molecule

For most of the cell cycle, chromosomes consist of unreplicated DNA molecules. Before cell division, each long molecule of double stranded DNA must be replicated.

For this to happen, it is first untwisted and separated (unzipped) at high speed at its replication fork by an enzyme called **helicase**. Another enzyme relieves the strain that this generates by cutting, winding and rejoining the DNA strands.

Step 2
Making new DNA strands

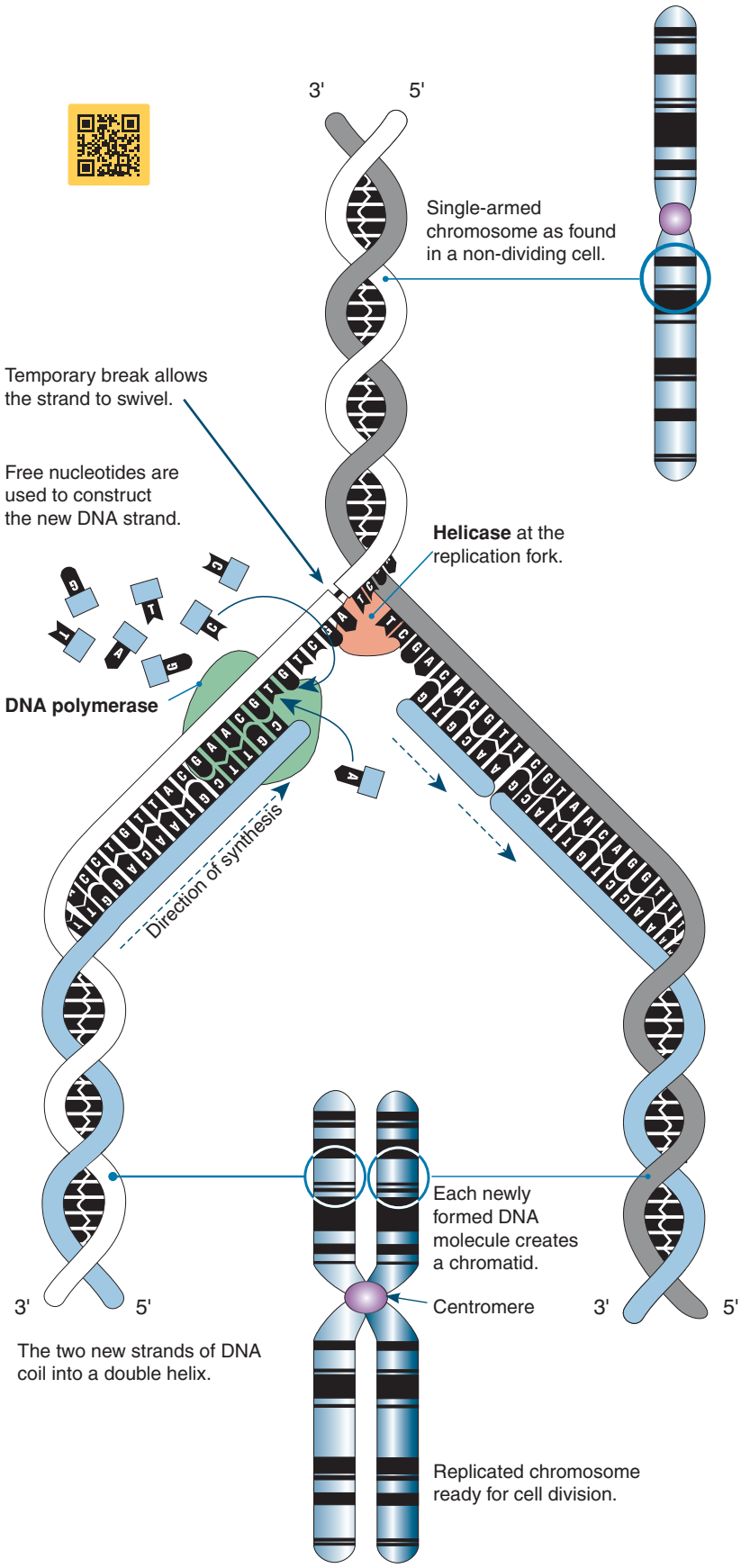
The formation of new DNA is carried out mostly by an enzyme complex called **DNA polymerase**.

DNA polymerase catalyses the condensation reaction that joins adjacent nucleotides. The strand is synthesised in a 5' to 3' direction, with the polymerase moving 3' to 5' along the strand it is reading. Thus the nucleotides are assembled in a continuous fashion on one strand but in short fragments on the other strand. These fragments are later joined by an enzyme to form one continuous length.

Step 3
Rewinding the DNA molecule

Each of the two new double-helix DNA molecules has one strand of the original DNA (dark grey and white) and one strand that is newly synthesised (blue). The two DNA molecules rewind into their double-helix shape again.

DNA replication is semi-conservative, with each new double helix containing one old (parent) strand and one newly synthesised (daughter) strand. The new chromosome has twice as much DNA as a non-replicated chromosome. The two chromatids will become separated in the cell division process to form two separate chromosomes.



ISBN: 978-1-98-856655-9

1. What is the purpose of DNA replication? _____

2. Summarise the three main steps involved in DNA replication:

   (a) _____

   (b) _____

   (c) _____

3. For a cell with 22 chromosomes, state how many chromatids would exist, following DNA replication: _____

4. What percentage of DNA in each daughter cell is new, and what percentage is original? _____

5. What does it mean when we say DNA replication is semi-conservative? _____

6. How are the new strands of DNA lengthened during replication? _____

7. What rule ensures that the two new DNA strands are identical to the original strand? _____

8. Why does one strand of DNA need to be copied in short fragments? _____

9. Match the statements in the table below to form complete sentences, then put the sentences in order to make a coherent paragraph about DNA replication and its role:

The enzymes also proofread the DNA during replication...	...is required before mitosis or meiosis can occur.
DNA replication is the process by which the DNA molecule...	...by enzymes.
Replication is tightly controlled...	...to correct any mistakes.
After replication, the chromosome...	...and half new DNA.
DNA replication...	...during mitosis.
The chromatids separate...	...is copied to produce two identical DNA strands.
A chromatid contains half original ...	...is made up of two chromatids.

Write the complete paragraph here:

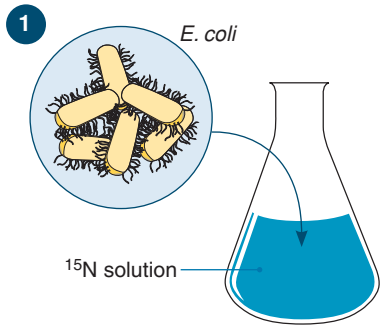
ISBN: 978-1-98-856655-9

50 Modelling DNA Replication

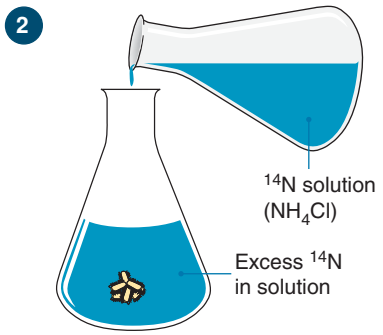
Key Idea: Meselson and Stahl devised an experiment that showed DNA replication is semi-conservative.

Three models were proposed to explain how DNA replicated. Watson and Crick proposed the **semi-conservative model** in which each DNA strand served as a template, forming a new DNA molecule that was half old and half new DNA. The conservative model proposed that the original DNA served as a complete template so that the resulting DNA was completely new. The dispersive model proposed that the two new DNA molecules had part new and part old DNA interspersed throughout them. In 1958 two scientists, Matthew Meselson and Franklin Stahl, carried out an experiment that showed how DNA replicates. Meselson and Stahl grew bacteria in a solution containing a heavy nitrogen isotope (^{15}N) until their DNA contained only ^{15}N. The superscript number = the nitrogen mass. The bacteria were then placed into a growth solution containing the nitrogen isotope (^{14}N), which is lighter than ^{15}N. After a set number of generation times, the DNA was extracted and centrifuged in a solution that provides a density gradient. Heavy DNA (containing only ^{15}N) sinks to the bottom, light DNA (containing only ^{14}N) rises to the top, and intermediate DNA (one light and one heavy strand) settles in the middle. In this activity, you will model the Meselson and Stahl experiment and determine the mode of DNA replication by comparing your results to the results of Meselson and Stahl.

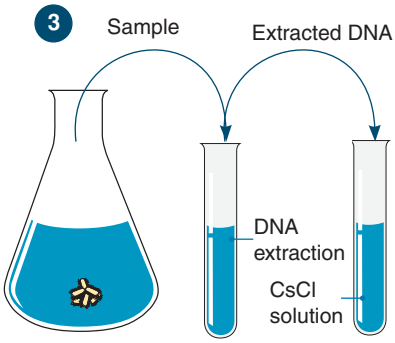
Meselson and Stahl's experiment



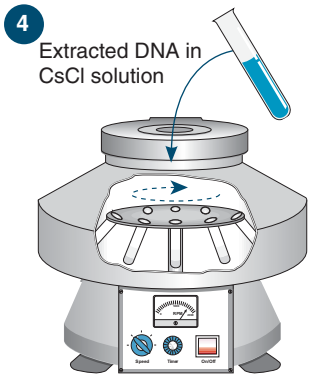
E. coli were grown in a nutrient solution containing ^{15}N. After 14 generations all the bacterial DNA contained ^{15}N. A sample was removed. This was **generation 0**.



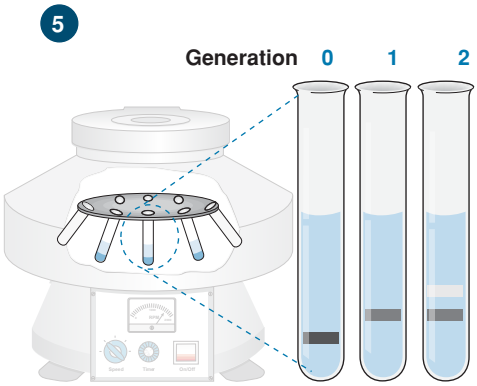
Generation 0 was added to a solution with excess ^{14}N (as NH_4Cl). During replication, new DNA would incorporate ^{14}N and be 'lighter' than the original DNA (which had only ^{15}N).



Each generation (~ 20 minutes), a sample was taken and treated to release the DNA. The DNA was placed in a CsCl solution which provided a density gradient for separating the DNA.



Samples were spun in a high speed ultracentrifuge at 140,000 *g* for 20 hours. Heavier ^{15}N DNA moved closer to the bottom of the test tube than light ^{14}N DNA or ^{14}N/ ^{15}N intermediate DNA.



All the DNA in the generation 0 sample moved to the bottom of the test tube. All the DNA in the generation 1 sample moved to an intermediate position. At generation 2, half the DNA was at the intermediate position and half was near the top of the test tube. In subsequent generations, more DNA was near the top and less was in the intermediate position.

Models for DNA replication

Conservative | Semi-conservative | Dispersive

1. Describe each of the DNA replication models:

   (a) Conservative: _____

   _____

   (b) Semi-conservative: _____

   _____

   (c) Dispersive: _____

   _____

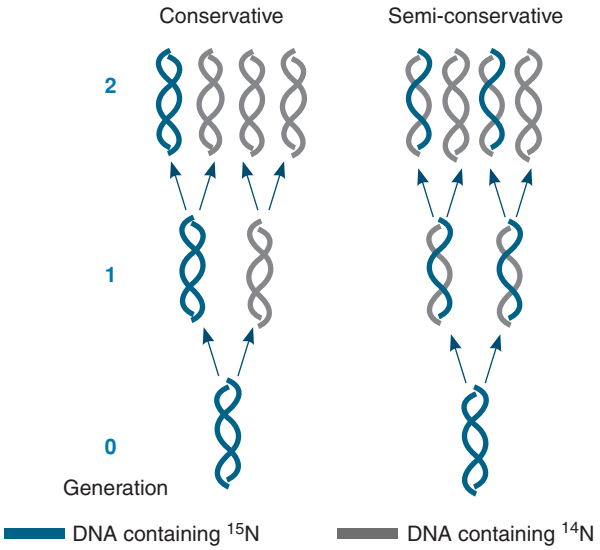
ISBN: 978-1-98-856655-9

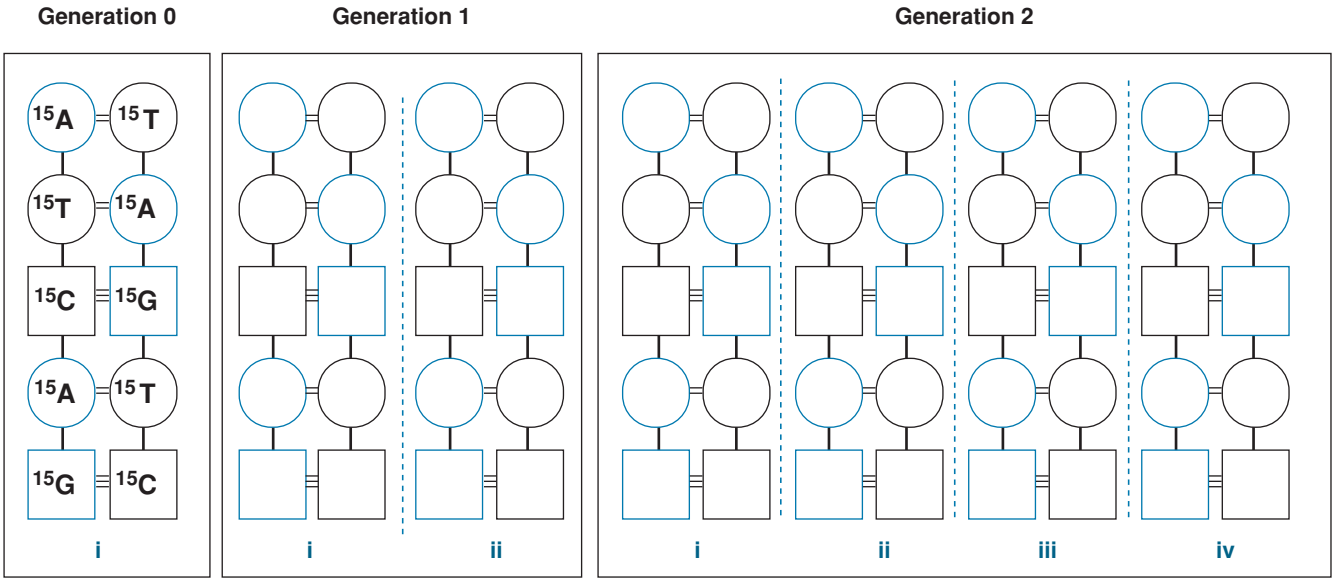
2. In this part of the activity you will model the **semi-conservative** model of DNA replication. 1(a) and (d (i)) are done for you:

   (a) The DNA model below represents the DNA of the bacteria after growing in the solution containing the heavy nitrogen (Generation 0). The relative mass of the DNA can be modelled by adding together the nitrogen masses.

   (b) In the space below (centre) (Generation 1), split the DNA from Generation 0 along its centre, then write in the complementary base pairs to form two DNA strands (i and ii). This represents the DNA after it has been grown in the solution containing ^{14}N for one generation.

   (c) In the space below (right) (Generation 2) split the two DNA chains along their centres, then write the complementary base pairs to form four new DNA strands (i, ii, iii, and iv). This represents the DNA after it has been growing in the solution containing the ^{14}N for two generations.





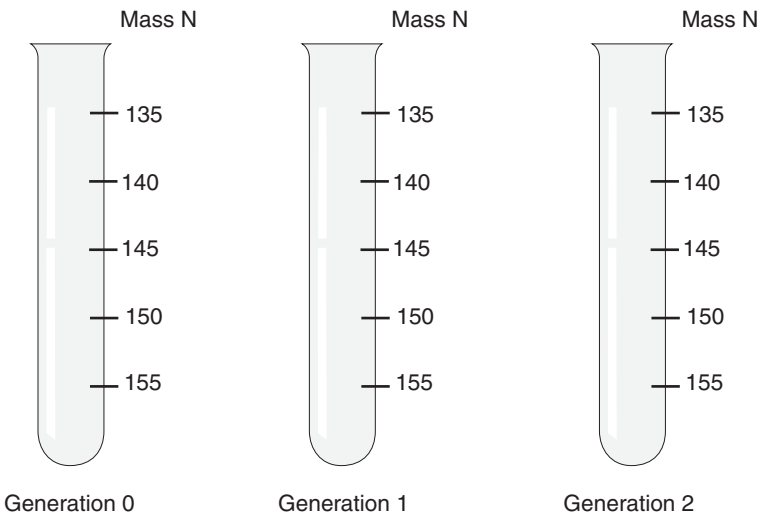
(d) Mass of nitrogen in DNA strand, Generation 0: i: 150

Mass of nitrogen in DNA strands, Generation 1: i: _____ ii: _____

Mass of nitrogen in DNA strands, Generation 2: i: _____ ii: _____

iii: _____ iv: _____

(e) On the test tubes (right), mark a bar representing the mass of each of the DNA strands from (a), (b), and (c).



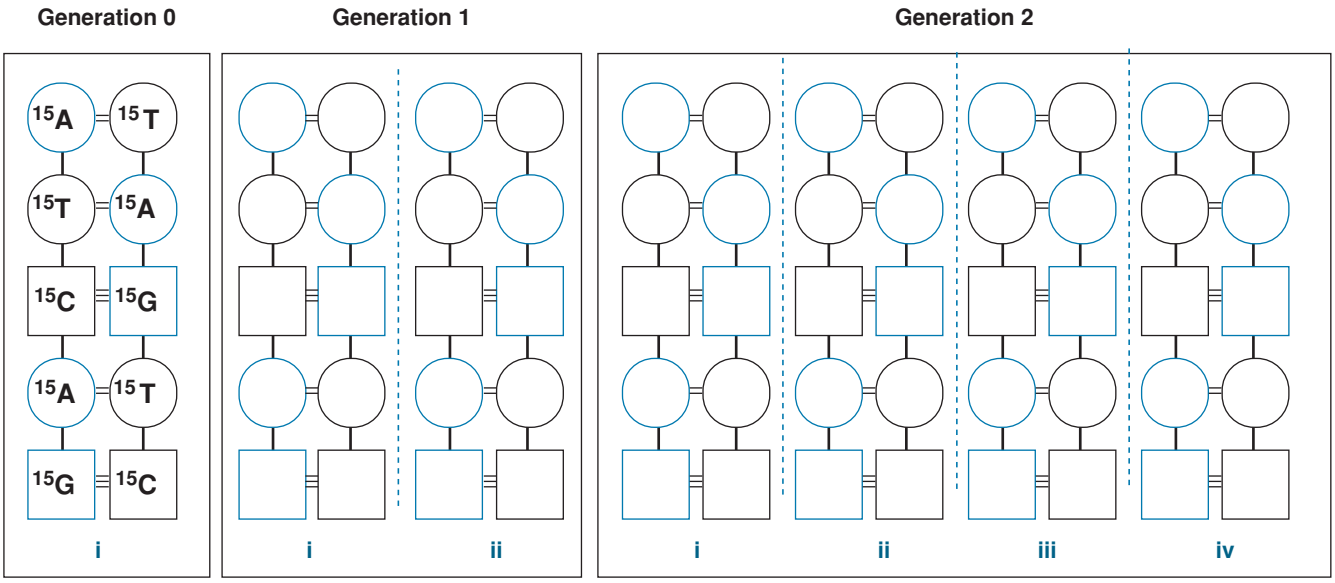
ISBN: 978-1-98-856655-9

3. In this part of the activity you will model the **conservative** model of DNA replication:

   (a) The DNA model below represents the DNA of the bacteria after it has grown in the solution containing the heavy nitrogen (**Generation 0**). The relative mass of the DNA can be modelled by adding together the nitrogen masses.

   (b) In the box below (centre), recreate the original DNA strand. Beside it, create a matching strand with bases using the light nitrogen. This represents the DNA after it has been replicated in the solution with the ^{14}N for one generation.

   (c) In the space below (right), recreate the original DNA strand and the first generation strand. Beside these, create matching strands with bases using the ^{14}N. This represents the DNA after it has been growing in the solution containing the ^{14}N for two generations.



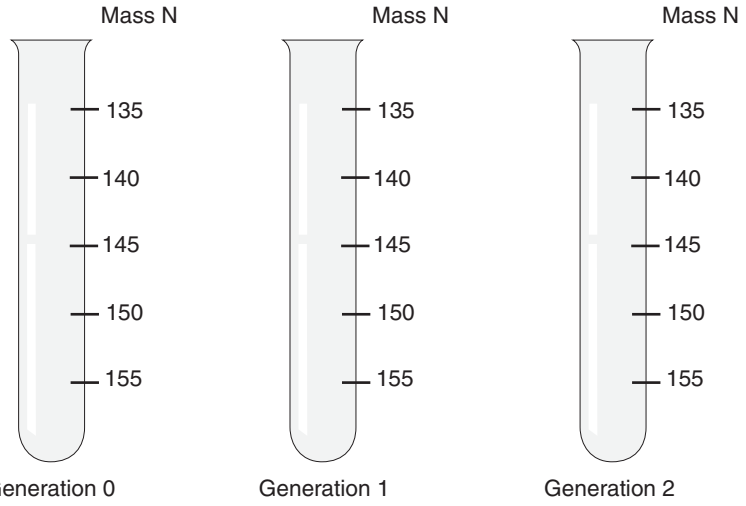
   (d) Mass of nitrogen in DNA strand, Generation 0: i: _____

   Mass of nitrogen in DNA strands, Generation 1: i: _____ ii: _____

   Mass of nitrogen in DNA strands, Generation 2: i: _____ ii: _____

   iii: _____ iv: _____

   (e) On the appropriate test tubes (right), mark a bar representing the mass of each of the DNA strands from Generation 0, Generation 1, and Generation 2



4. In their experiment, Meselson and Stahl obtained the following results: **Generation 0** = 100% 'heavy DNA'; **Generation 1**= 100% 'intermediate DNA'; **Generation 2** = 50% 'intermediate DNA', 50% 'light DNA'.

   From the results of your two modelling exercises, decide which matches the result of Meselson and Stahl. How does DNA replicate (conservatively or semi-conservatively)?

   _____

   _____

ISBN: 978-1-98-856655-9

51 Cell Replication and the Continuity of Species

Key Idea: Genetic continuity ensures genetic information is preserved across generations, both in new cells and new organisms.

In asexually reproducing species, e.g. bacteria or yeast, genetic information is preserved from generation to generation by mitotic cell divisions. In sexually reproducing species, meiotic cell division produces gametes which carry half the genetic information as one set of chromosomes, N. When the male and female gametes unite, the full set of chromosomes is restored, as 2N. This continuity of genetic information from cell to cell and generation to generation ensures cells have the genetic information they need to survive and remain compatible with the all the cells in an organism, and with members of the same species.

Mitosis and continuity

- Mitosis produces cells with identical genetic information. For multicellular organisms this is important because it ensures that cells in the organism have the same genetic information, and that cells in a tissue, e.g. muscle tissue, all grow and behave in the same way.

Cells maintain genetic continuity by mitosis.

Sexually reproducing species maintain genetic continuity by meiosis.

Meiosis and continuity

- If two cells of the same species unite to produce a new cell they will both add their full sets of chromosomes to the newly formed cell. If this happens, the new cell will have twice the original number of chromosomes, and so will behave differently to the original cells. Meiosis is the process used by sexually reproducing organisms to produce gametes carrying one set of chromosomes (half the genetic information of the original cell). This ensures that when the gametes unite the correct number of chromosomes for the organism is restored.

Mutation and continuity

- Mutations cause changes in DNA. If the mutation occurs in a mitotically dividing cell the daughter cells may behave differently to the parent cell. This could have negative effects on an organism's tissues. For example cancer (right) occurs when the DNA encoding the regulation of cell division has been mutated.
- Mutations that occur in gametes are passed onto the offspring. Mutations produce new diversity between parents and offspring. Mutations that help offspring survive and reproduce are retained; those that have negative effects on the offspring are often not passed on. This helps the species evolve and adapt to changes in the environment, with useful changes in DNA being retained in the population.
- As useful mutations accumulate over generations, the species changes. Although the genetic lineage may be continuous over generations, daughter species many generations removed may have changed so much from the original parent species that they would not be compatible if brought together again.
- Occasionally, mutations in the gametes may produce large changes in the DNA, e.g. missing or duplicated chromosomes. In this case, the offspring may not survive, or become instantly incompatible with the rest of the species. This may produce an instantaneous speciation event.

Cancer cells

Over generations species change as useful mutations accumulate

1. Explain how mitosis ensures genetic continuity between generations of cells: _____

2. How does meiosis maintain genetic continuity between parents and offspring? _____

3. How do mutations allow a species to adapt over time while maintaining genetic continuity? _____

ISBN: 978-1-98-856655-9

52 Chapter Review: Did You Get It?

1. State three reasons why cells divide: _____

2. How many chromosomes are in the daughter cells after mitosis in cells with the following number of chromosomes:

   (a) 22: _____ (b) 48: _____

3. How many chromosomes are in the daughter cells after meiosis in cells with the following number of chromosomes:

   (a) 22: _____ (b) 48: _____

4. (a) Label the cell cycle (right) with the following labels: G_1, G_2, M, S, cytokinesis.

   (b) Briefly describe what happens in each of the following phases:

   G_1: _____

   _____

   _____

   G_2: _____

   _____

   _____

   M phase: _____

   _____

   S phase: _____

   _____

5. (a) Which base does the base thymine (T) pair with in DNA? _____

   (b) Which base does the base cytosine (C) pair with in DNA? _____

6. In the boxes below draw and label a series of diagrams to illustrate how a strand of DNA replicates:

DNA unzips.	Nucleotides are added to the parent strands.	The parent and daughter strands rewind to form two new DNA strands.

7. What effect does crossing over in meiosis have on the gametes produced? _____

   _____

   _____

ISBN: 978-1-98-856655-9

CHAPTER 3

DNA and Polypeptide Synthesis

Key terms

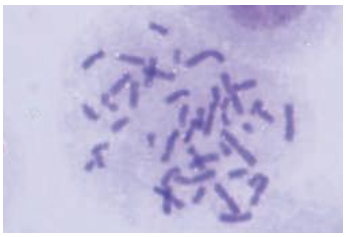
allele
amino acid
anticodon
autosome
chromosome
codon
epigenetics
exon
gene
genome
genotype
homologous chromosomes
intron
phenotype
plasmid
polypeptide
primary structure
protein
quaternary structure
secondary structure
sex chromosome
tertiary structure
transcription
translation

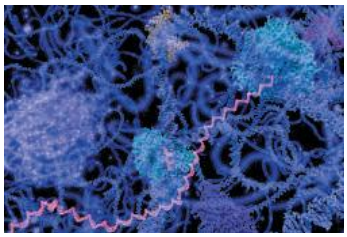
Activity number

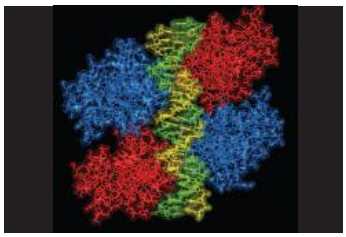
Inquiry question: *Why is polypeptide synthesis important?*

Chromosomes, genes and genomes

Key skills and knowledge







Gene expression

Key skills and knowledge

Influence of environment on gene expression

Key skills and knowledge

Protein structure and function

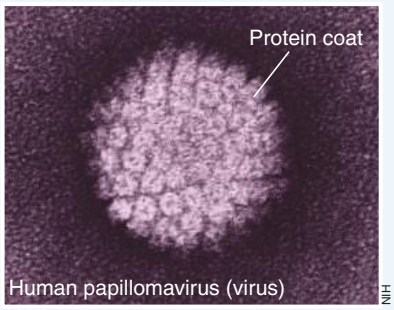
Key skills and knowledge

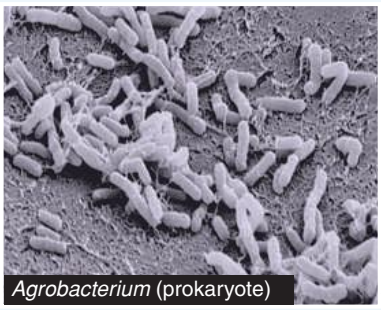
53 Genomes, Genes, and Alleles

Key Idea: A genome is the entire haploid amount of genetic material, including all the genes, of a cell or organism. Eukaryotes can have different versions of a gene (alleles) because they have two copies of each gene.

The **genome** refers to all the genetic material in one haploid set of chromosomes. The genome contains all of the information the organism needs to function and reproduce. Every cell in an individual has a complete copy of the genome. Within the genome are sections of DNA, called **genes**, which code for proteins. Collectively, genes determine what an organism looks like (its traits). Eukaryotes have two copies of each gene, one inherited from each parent, so it is possible for one individual to have two different versions of a gene. These different versions are called **alleles**.

The location and size of the genome varies between organisms





Genome size — Small → Large

Number of genes — Few → Many

The viral genome is contained within the virus's outer protein coat. Viral genomes are typically small and highly variable. They can consist of single stranded or double stranded DNA or RNA and contain only a small number of genes.

The human papillomavirus (HPV) genome is a double stranded circular DNA molecule ~8000 bp long.

In prokaryotes, most of the DNA is located within a single circular chromosome, which makes them haploid, i.e. having one allele, for most genes. Many bacteria also have small accessory chromosomes called plasmids, which carry genes for special functions such as antibiotic resistance and substrate metabolism.

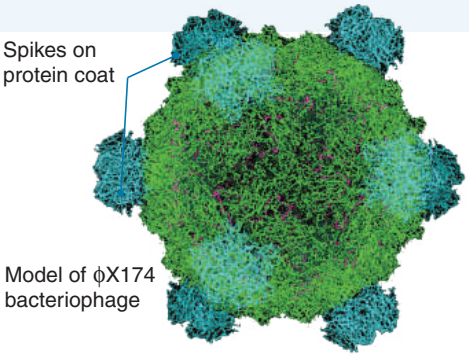
The *Agrobacterium* genome (5.7 Mb long) is unusual. It consists of a circular chromosome, a linear chromosome, and two plasmids. Linear chromosomes are very rare in bacteria.

In eukaryotes, most of the DNA is located inside the cell's nucleus. A small amount exists in the chloroplasts (in plants) and in mitochondria. The DNA is arranged into linear chromosomes and most eukaryotes are diploid, with two sets of chromosomes, one from each parent.

The koala genome is ~3.37 Mb long in 8 chromosomes (the diploid number is 16). This genome size is similar to humans.

Measuring genomes

- Genome size is often expressed as the number of base pairs (bp). The unit most often used to show the size of a genome is the megabase (Mb). Note: 1 megabase = 1 million base pairs.
- The image on the right shows the ϕX174 bacteriophage, a virus that infects bacterial cells. Its entire genome is only 5375 bp long (0.005375 Mb) and it contains only nine genes, coding for nine different proteins. At least 2000 times this amount of DNA would be found in a single bacterial cell; 500,000 times that quantity would be found in the genome of a single human cell.



1. Define the following terms:

   (a) Genome: _____

   (b) Gene: _____

   (c) Allele: _____

2. Describe the general trend for genome size and gene number for viruses, bacteria, and eukaryotic organisms:

   _____

3. Explain why an individual eukaryote can have different versions of a gene (allele) but viruses and bacteria do not:

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

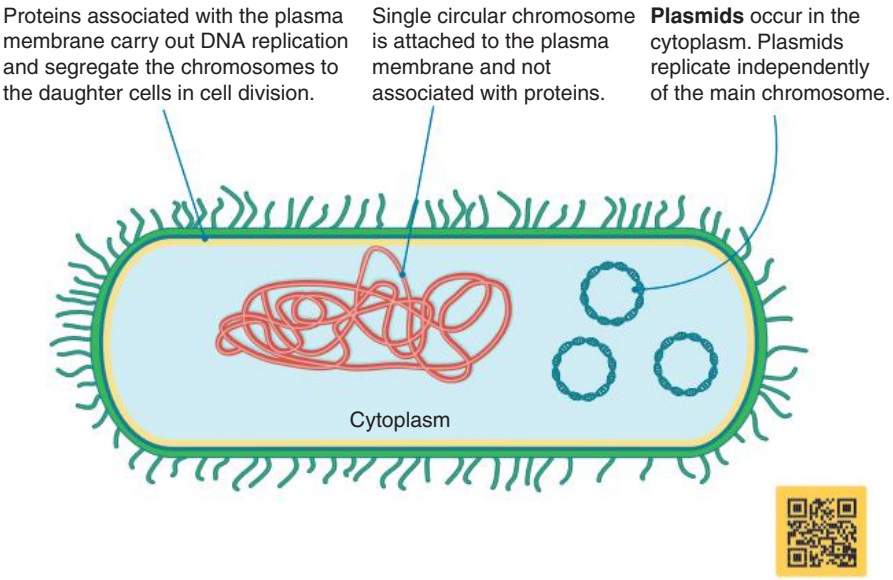
54 Prokaryotic Chromosomes

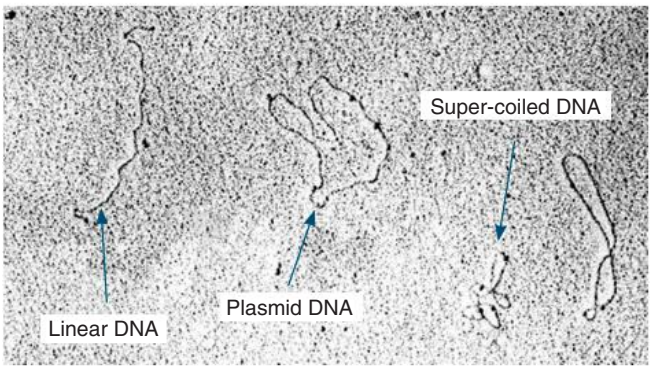
Key Idea: Prokaryotic DNA is packaged as a single circular chromosome. Smaller plasmids may be included.

DNA is a universal carrier of genetic information, but it is packaged differently in prokaryotic and eukaryotic cells. Unlike eukaryotic chromosomes, the prokaryotic chromosome is not enclosed in a nuclear membrane and is not associated with protein. It is a single circular (rather than linear) molecule of double stranded DNA, attached to the plasma membrane. It is located in a nucleoid region which is in direct contact with the cytoplasm. As well as the bacterial chromosome, bacteria often contain small, circular, double-stranded DNA molecules called plasmids. Plasmids are independent of the main bacterial chromosome and usually contain 5-100 genes that are not crucial to cell survival under normal conditions.

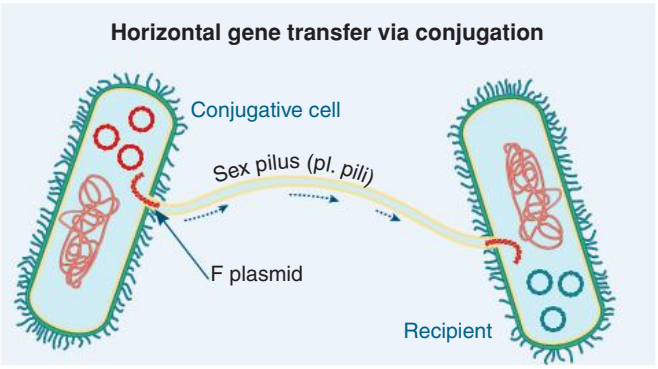
The prokaryotic genome

- In contrast to eukaryotes, prokaryotic DNA consists almost entirely of protein coding genes and their regulatory sequences. It was the study of prokaryotic genomes that gave rise to the **one gene-one protein hypothesis**, which still holds true for bacteria.
- The chromosomal DNA is located in a **nucleoid region**. It is not enclosed in a membrane. The nucleoid may take up as much as 20% of the cell's volume in actively growing cells.
- Most bacteria have a single, circular chromosome. This makes them haploid for most genes, unless copies are located on **plasmids** (small, circular, auxiliary DNA strands). Plasmids are generally circular but can also exist as linear, or supercoiled structures.





Plasmids vary in size from 1000 base pairs (bp) to hundreds of thousands of base pairs. In bacteria, they play an important role in providing extra genetic material, conferring extra properties such as antibiotic resistance. Plasmids can be transferred between bacterial cells in the process of plasmid transfer called conjugation (right).



Conjugation: Special conjugative (F) plasmids contain transfer genes, which enable conjugation and the transfer of genetic information between bacterial cells. Transfer occurs via a sex pilus, which briefly connects the donor and the recipient. Conjugation provides another route for acquiring genes, in addition to transfer via binary fission.

1. State three important ways in which prokaryotic chromosomes differ from eukaryotic chromosomes:

   (a) _____

   (b) _____

   (c) _____

2. Explain the consequences to protein synthesis of the prokaryotic chromosome being free in the cytoplasm: _____

   _____

   _____

3. Most of the bacterial genome comprises protein coding genes and their regulatory sequences. What is the consequence of this to the relative sizes of bacterial and eukaryotic genomes?

   _____

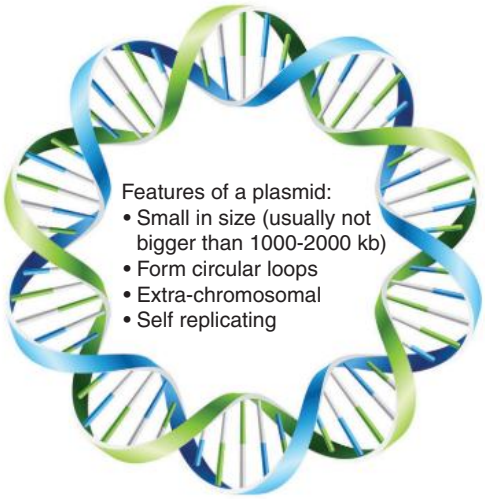
   _____

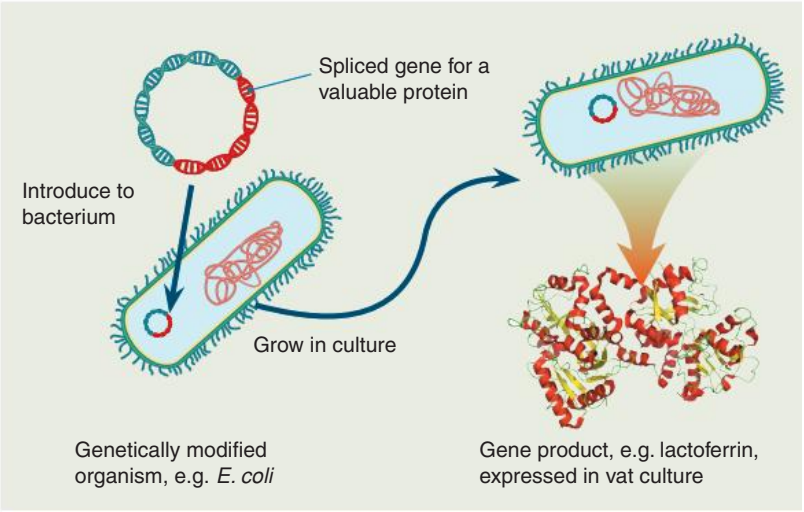
ISBN: 978-1-98-856655-9

55 Plasmid DNA

Key Idea: Plasmids play a special role in storing DNA in prokaryotes. Plasmids can be exploited for gene manipulation.

Prokaryotes store most of their genetic information in one large chromosome but a small proportion can commonly be found as independently replicating, circular, extra-chromosomal pieces of DNA known as **plasmids**. Plasmids may carry important genes, such as those for the production of toxins that eliminate prokaryote competitors. Plasmids are less common in eukaryotes but some species, such as the yeast *Saccharomyces,* do have them. The genetic material from viruses may form plasmid-like structures called episomes once they have infected a cell.

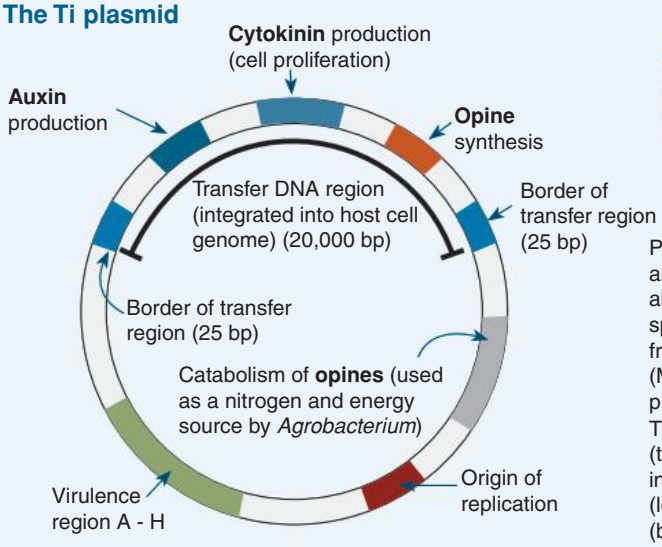




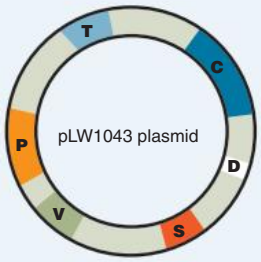
The properties of plasmids enable them to be used as tools to introduce genetic material into organisms. A gene is spliced into a plasmid and the plasmid is inserted into a recipient organism, e.g. a bacterium. The recipient will then produce the gene product as part of its normal metabolism. This technique has enabled the industrial-scale production of valuable gene products, such as human proteins, from bacteria.

The make-up of plasmids

The Ti plasmid



The pLW1043 plasmid



Genes for

- **C** Conjugative ability
- **T** Trimethoprim resistance
- **V** Vancomycin resistance
- **D** Disinfectant resistance
- **S** Streptomycin resistance
- **P** Penicillin resistance

Plasmids often carry genes for beneficial traits, including antibiotic resistance and the ability to use new substrates. The ability of bacteria to exchange plasmids has contributed to the spread of antibiotic resistance. The pLW1043 plasmid (above) from the superbug, Methicillin Resistant *Staphylococcus aureus* (MRSA) carries several genes for antibiotic resistance, acquired progressively over time.

The bacterium *Agrobacterium tumefaciens* often contains the Ti (tumor inducing) plasmid. This plasmid is able to transfer genes into plant cells and cause disease. Several regions on the plasmid (left) help it to infect plants. The plasmid is just over 200,000 bp (base pairs) long and contains 196 genes. The mapping of its genes has made it of great importance in creating genetically modified plants.

1. What is a plasmid? _____

2. Explain how a plasmid can convey a survival advantage to bacteria under certain conditions: _____

_____

_____

3. (a) Why are plasmids (such as the Ti plasmid) useful to genetic engineers? _____

_____

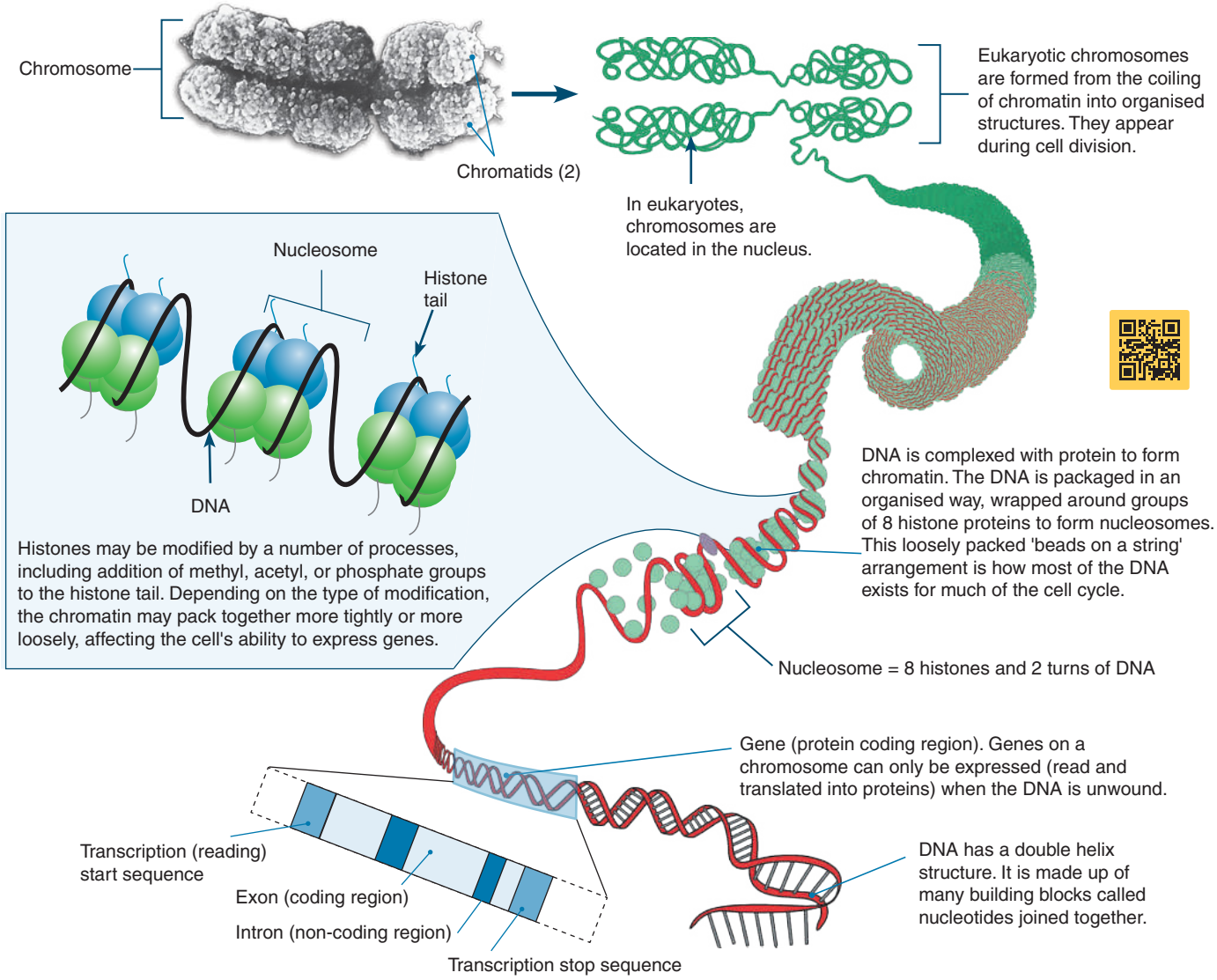
(b) Into which region of the Ti plasmid would you insert a gene in order for it to be transferred into a host plant cell?

_____

ISBN: 978-1-98-856655-9

56 Eukaryotic Chromosomes

Key Idea: In eukaryotes DNA is stored as linear chromosomes. The DNA in eukaryotes is packaged as discrete linear chromosomes. The number of chromosomes varies from species to species. The extent of DNA packaging changes during the life cycle of the cell, but classic chromosome structures (below) appear during metaphase of mitosis.



1. Explain why eukaryotic DNA needs to be packaged up to fit inside a cell nucleus: _____

2. How do histone proteins help in the coiling up of DNA? _____

3. Suggest why a cell coils up its chromosomes into tight structures when it is going to divide: _____

4. Explain how the packaging of DNA in an organised way enables closer regulation of gene expression: _____

ISBN: 978-1-98-856655-9

Sex chromosomes and autosomes

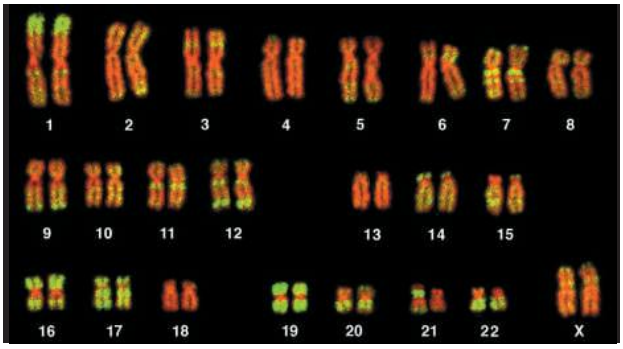
- In eukaryotes, the chromosomes are located within the cell's nucleus. Sexually reproducing organisms usually have two sets of chromosomes (they are diploid). One set is inherited from each parent at the time of fertilisation. The corresponding chromosomes from each parent form a pair called a **homologous pair** (**homologous chromosomes)**. Chromosomes can be classified either as sex chromosomes or autosomes.

Sex chromosomes

- **Sex chromosomes** determine the sex of organisms and the genes they carry are called sex linked.
- Humans have two sex chromosomes (X and Y). Egg cells contain 22 autosomes and an X chromosome. Sperm cells contain 22 autosomes and either an X or Y chromosome. Humans have one pair of sex chromosomes: XX = females. XY = males.
- Sex chromosomes are not homologous. They differ in height, banding pattern, and centromere position. The X chromosome is much larger than the Y chromosome and contains more genes (above right). Genes on the X chromosome that are not present on the Y chromosome will always be expressed in males.

Autosomes

- Most chromosomes are **autosomes** (non-sex chromosomes). Their genes determine most phenotypic characteristics (but not sex).
- Human cells have 22 pairs of autosomes (1-22 below). The pairs are homologous so the chromosomes in each pair have similar heights, banding patterns, and centromere positions. They also carry the same genes, in the same location.



Bolzer et al PLOS (2005) cc 4.0

5. Summarise the differences between sex chromosomes and autosomes under the headings below:

   (a) Function:

   i) Autosome: _____

   ii) Sex chromosome: _____

   (b) Are the chromosomes homologous (Yes/No)?

   i) Autosomes: _____ ii) Sex chromosomes: _____

   (c) Number/pairs of chromosomes in humans:

   i) Autosomes: _____ ii) Sex chromosomes: _____

   (d) Are the genes sex linked (Yes/No)?

   i) Autosomes: _____ ii) Sex chromosomes: _____

6. Chromosomes are often depicted in different ways, in different sources. Search the internet for some of these depictions, print them out, and paste them into the space below. In what ways are these depictions accurate or inaccurate?

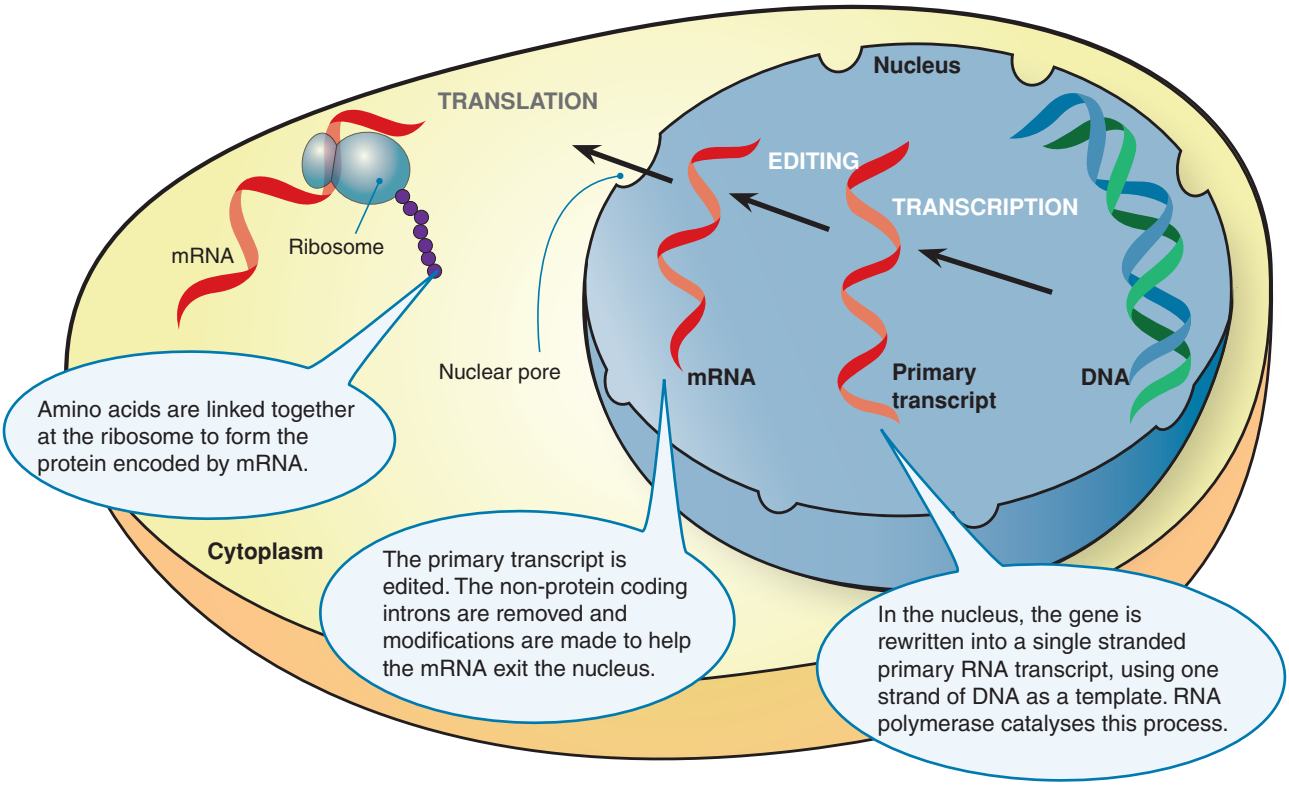
ISBN: 978-1-98-856655-9

57 What is Gene Expression?

Key Idea: Genes are sections of DNA that code for proteins. Genes are expressed when they are transcribed into messenger RNA (mRNA) and then translated into a protein.

Gene expression is the process by which the information in a gene is used to synthesise a protein. It involves **transcription** of the DNA into mRNA and **translation** of the mRNA into protein. Eukaryotic genes include non-protein coding regions called introns. These regions of intronic DNA must be edited out before the mRNA is translated by the ribosomes. Transcription of the genes and editing that primary transcript to form the mature mRNA occurs in the nucleus. Translation of the protein by the ribosomes occurs in the cytoplasm.

A summary of eukaryotic gene expression



1. What is a gene? _____

_____

2. (a) What does gene expression mean? _____

_____

(b) What are the three stages in gene expression in eukaryotes and what happens in each stage?

i. _____

ii. _____

iii._

3. The photograph (right) shows a scanning electron micrograph (SEM) of a giant polytene chromosome. These chromosomes are common in the larval stages of flies, which must grow rapidly before changing to the adult form. They form as a result of repeated cycles of DNA replication without cell division, which creates many copies of genes. Within these chromosomes, visible 'puffs' indicate regions where there is active transcription of the genes.

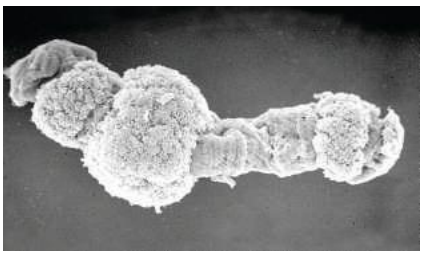
(a) What is the consequence of active transcription in a polytene chromosome?

_____

(b) Why might this be useful in a larval insect? _____

_____

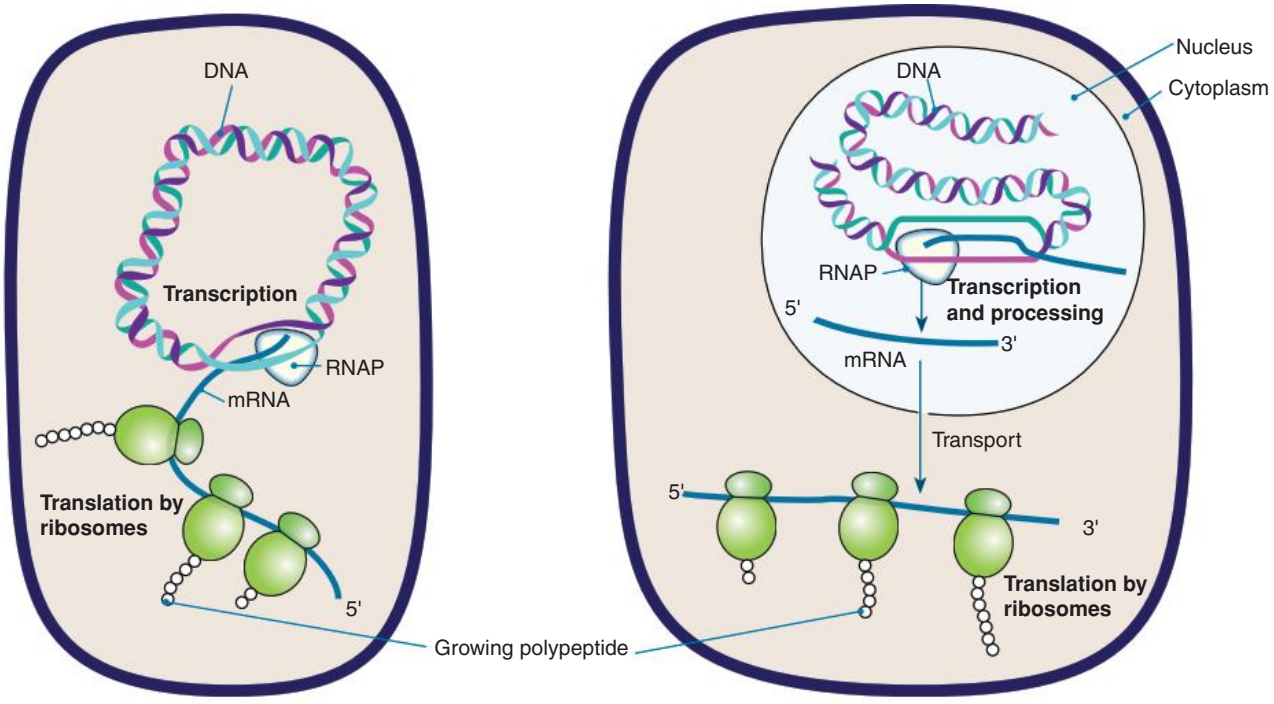
_____



ISBN: 978-1-98-856655-9

Comparing gene expression in prokaryotes and eukaryotes

In both prokaryotes and eukaryotes, genes are transcribed by RNA polymerase (**RNAP**) and translated by ribosomes. However, there are some important differences. In eukaryotes, primary RNA must be edited and processed before passing from the nucleus to the cytoplasm. In prokaryotes, there is no nucleus and ribosomes can begin translating a gene while it is still being transcribed.



Prokaryote **Eukaryote**

4. For the following triplets on a DNA template strand, state the codon sequence for the mRNA that would be synthesised:

   Triplets on the DNA: T A C T A G C C G C G A T T T

   Codons on the mRNA: _____

   Triplets on the DNA: T A C A A G C C T A T A A A A

   Codons on the mRNA: _____

5. What is the significance of the promoter and terminator regions on the DNA? _____

   _____

6. Why might a cell employ several RNA polymerases to produce multiple RNA transcripts of a gene at any one time?

   _____

   _____

7. Based on the diagram above, describe two differences between gene expression in prokaryotes and eukaryotes:

   (a) _____

   _____

   (b) _____

   _____

8. Based on the diagram above, how is gene expression in prokaryotes and eukaryotes similar? _____

   _____

9. What benefit might there be to the way in which prokaryotes can begin translation while a gene is still being transcribed?

   _____

   _____

ISBN: 978-1-98-856655-9

58 What is the Genetic Code?

Key Idea: The genetic code is the set of rules by which the genetic information in DNA or mRNA is translated into proteins.

The genetic information for the assembly of amino acids is stored as three-base sequences. These three letter codes on mRNA are called **codons**. Each codon represents one of 20 amino acids used to make proteins. The code is effectively universal, being the same in all living things (with a few minor exceptions). The genetic code is summarised in a mRNA-amino acid table which identifies the amino acid encoded by each mRNA codon. The code is degenerate, meaning there may be more than one codon for each amino acid. Most of this degeneracy is in the third nucleotide of a codon.

The mRNA - amino acid table

The table on the right is used to 'decode' the genetic code. It shows which amino acid each mRNA codon codes for. There are 64 different codons possible; 61 code for amino acids, and three are stop codons.

Amino acid names are written as three letter abbreviations (e.g. Ser = serine). To work out which amino acid a codon codes for, carry out the following steps:

i Find the first letter of the codon in the row on the left hand side of the table. AUG is the start codon.

ii Find the column that intersects that row from the top, second letter, row.

iii Locate the third base in the codon by looking along the row on the right hand side that matches your codon.

e.g. **GAU** codes for Asp (aspartic acid)

Read second letter here

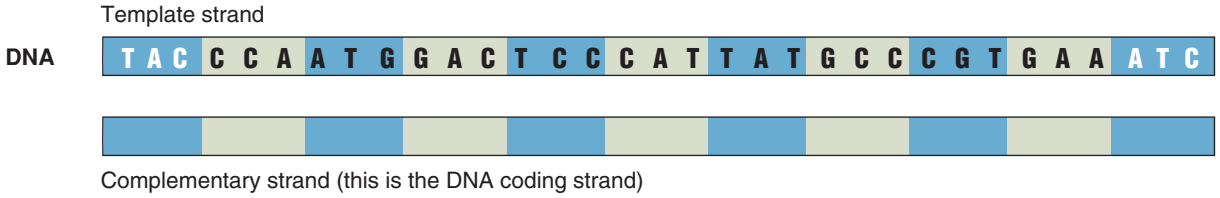
First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

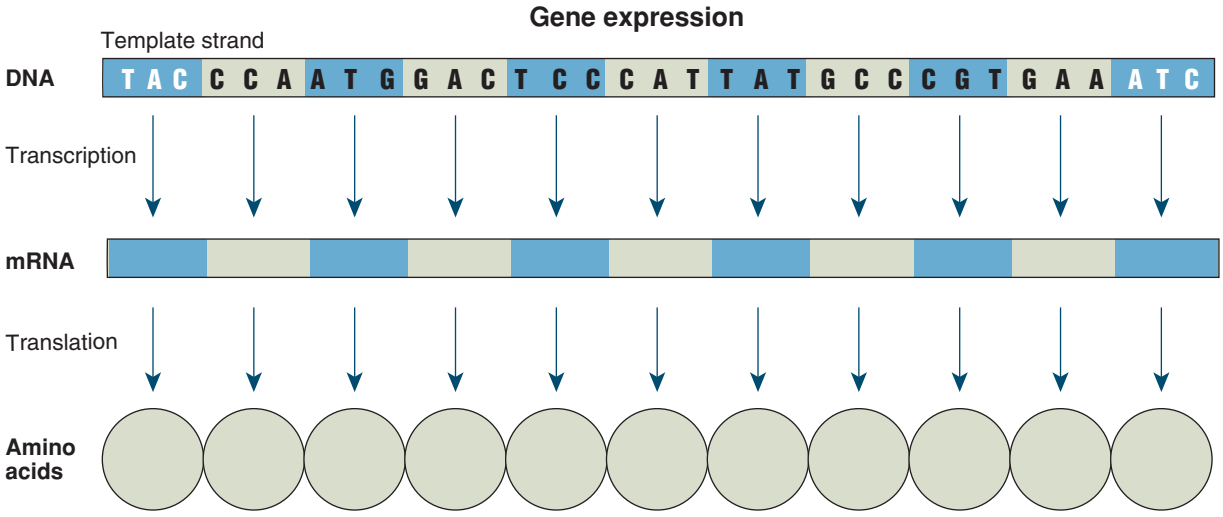
Read first letter here

Read third letter here

1. (a) Use the base-pairing rule to create the complementary strand for the DNA template strand shown below.

   (b) For the same DNA template strand, determine the mRNA sequence and use the mRNA-amino acid table to determine the amino acid sequence. Note that in mRNA, uracil (U) replaces thymine (T) and pairs with adenine.





2. What do you notice about the sequence on the DNA coding strand and the mRNA strand? _____

_____

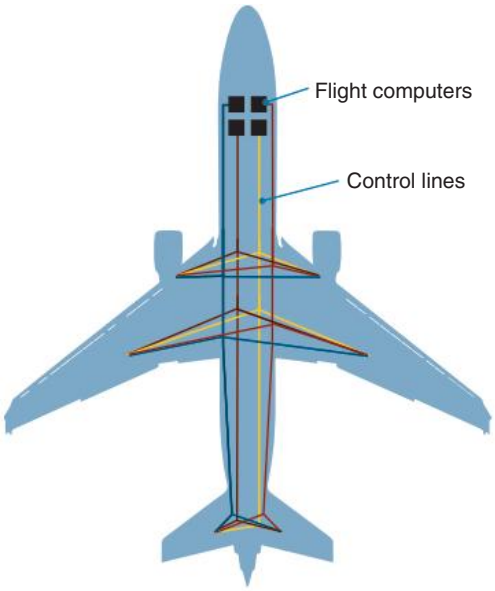
ISBN: 978-1-98-856655-9

Redundancy and degeneracy

Redundancy and degeneracy are important concepts in understanding the **genetic code.**

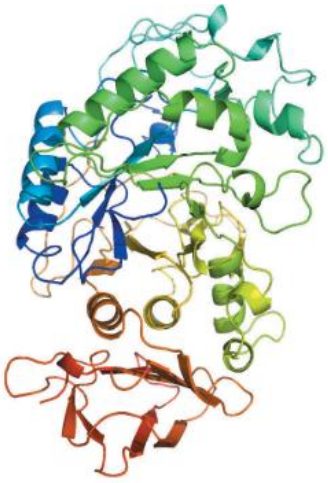
- **Redundancy** is when several situations code for, or control, the actions of one specific thing.
- **Degeneracy** is when a particular output can be produced by several different pathways.

Examples of redundancy and degeneracy are illustrated below. In modern aircraft redundant features add safety by making sure that if one system fails others will ensure a smooth, safe flight. Degeneracy can be seen in proteins when different proteins have the same function.



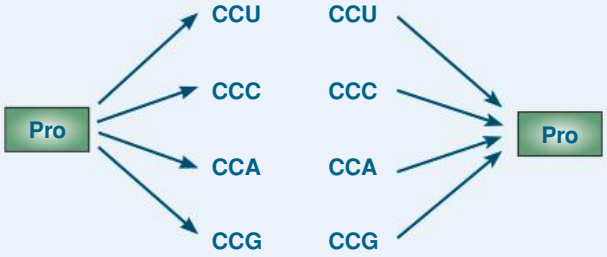
Modern aircraft (left) have multiple redundant features for safety. Often there are three or four flight computers linked independently to the flight surfaces and other input/output devices. If one computer or control line fails the others can continue to fly the plane normally.

Degeneracy is seen in the production of the enzymes salivary and pancreatic amylase. Salivary amylase breaks down carbohydrates in the mouth, whereas pancreatic amylase does so in the small intestine. The enzymes are encoded by different genes (AMY1A and AMY2A) but have the same functional role (right).



Salivary amylase (above) is structurally different to pancreatic amylase, but has the same function.

The genetic code shows **degeneracy**. This means that a number of 3 base combinations specify one amino acid. The codons for the same amino acid often differ by only a single letter (often the second or third). For example, proline is encoded by four different codons.



The degeneracy of the genetic code creates **redundancy**, so that several codons code for the same amino acid (e.g. CCU, CCC, CCA, and CCG code for proline). Note that although there is redundancy, there is no ambiguity - none of the codons encodes any other amino acid.

3. Explain how degeneracy adds 'safety' to the coding of protein chains: _____

4. The genetic code shows redundancy but no ambiguity. What does this mean and why is it important? _____

5. Identify the following:

   (a) The codons that encode valine (Val): _____

   (b) The codons that encode aspartic acid (Asp): _____

6. (a) Arginine (Arg) is encoded in how many ways? _____

   (b) Glycine (Gly) is encoded in how many ways? _____

   (c) Which amino acid(s) are encoded in only one way? _____

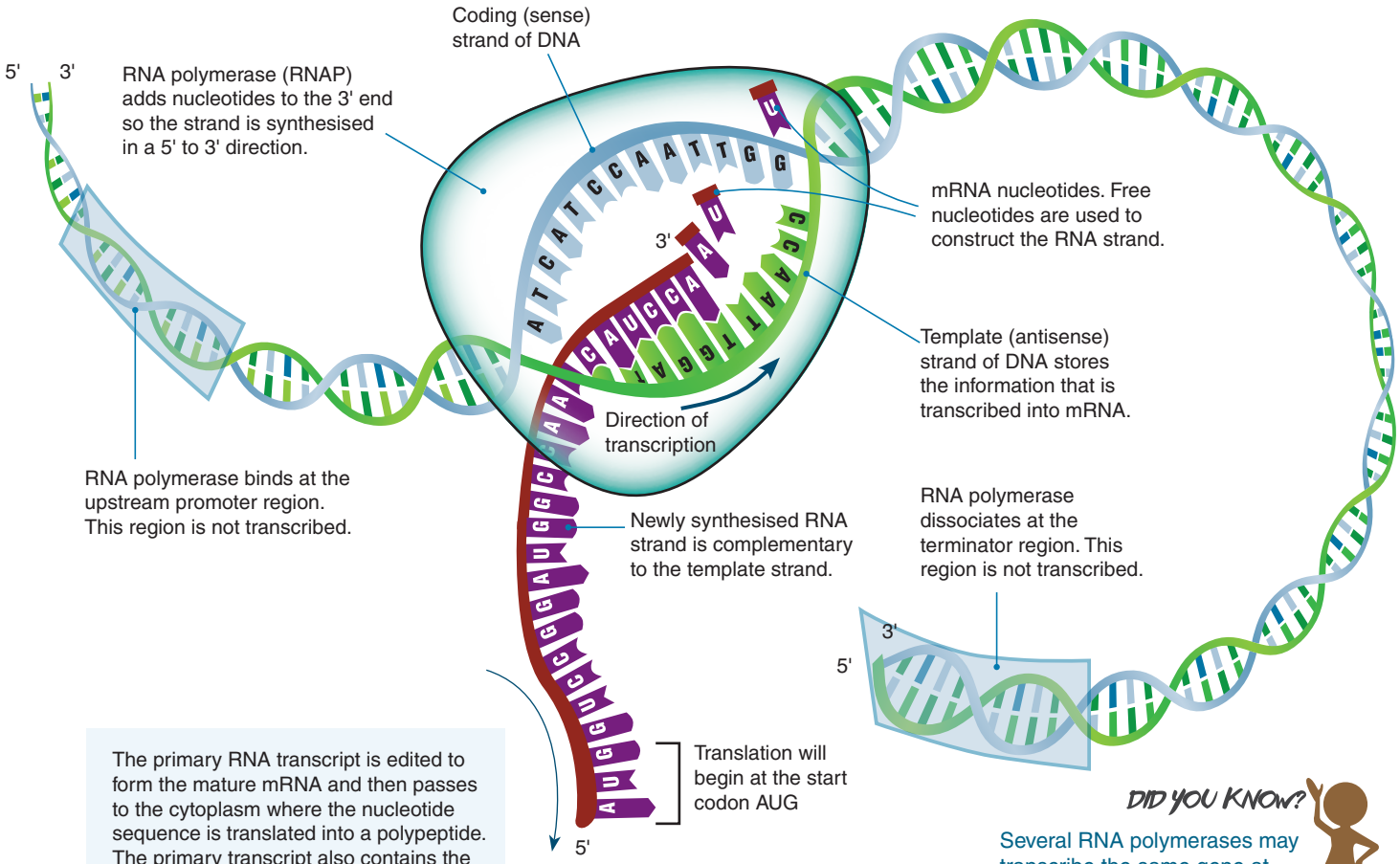
ISBN: 978-1-98-856655-9

59 Transcription in Eukaryotes

Key Idea: Transcription is the first step of gene expression. It involves the enzyme RNA polymerase rewriting the information into a primary RNA transcript. In eukaryotes, transcription takes place in the nucleus.

Transcription is the first stage of gene expression. It takes place in the nucleus and is carried out by the enzyme RNA polymerase. This enzyme rewrites the DNA into a primary RNA transcript using a single template strand of DNA. The protein-coding portion of a gene is bounded by a start (promoter) region and a terminator region. These regulatory regions control transcription by telling RNA polymerase where to start and stop transcription. In eukaryotes, non protein-coding sections called **introns** must first be removed and the remaining **exons** spliced together to form the mature mRNA before the gene can be translated into a protein. This editing process also occurs in the nucleus.

Transcription is carried out by RNA polymerase (RNAP)



The primary RNA transcript is edited to form the mature mRNA and then passes to the cytoplasm where the nucleotide sequence is translated into a polypeptide. The primary transcript also contains the 5' and 3' UTRs (untranslated regions), not shown here for reasons of clarity.

DID YOU KNOW?

Several RNA polymerases may transcribe the same gene at any one time, allowing a high rate of mRNA synthesis.

1. (a) Name the enzyme responsible for transcribing the DNA: _____

   (b) What strand of DNA does this enzyme use? _____

   (c) The code on this strand is the [same as / complementary to] the RNA being formed (circle correct answer):

   (d) Which nucleotide base replaces thymine in mRNA? _____

   (e) On the diagram, use a coloured pen to mark the beginning and end of the protein-coding region being transcribed.

2. (a) In which direction is the RNA strand synthesised? _____

   (b) Explain why this is the case: _____

3. (a) Why is AUG called the start codon? _____

   (b) What would the three letter code be on the DNA coding strand? _____

ISBN: 978-1-98-856655-9

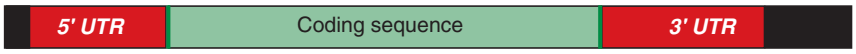
60 mRNA Processing in Eukaryotes

Key Idea: mRNA can undergo several types of modification after transcription to alter the protein produced.

Once a gene is transcribed, the primary transcript is modified to produce the mRNA strand that will be translated in the cytoplasm. Modifications to the 5' and 3' ends of the transcript enable the mRNA to exit the nucleus and remain stable long enough to be translated. Other post transcriptional modifications remove non-protein coding intronic DNA and splice exons in different combinations to produce different protein end products.

Primary RNA is modified by the addition of caps and tails

CAP
A guanine nucleotide cap at the 5' end of the primary transcript stops degradation during transport from the nucleus and helps in the first phase of translation.



POLY-A TAIL
Adenosine nucleotides are added to the primary transcript. These poly-A tails aid nuclear export, translation, and stability of the mRNA.

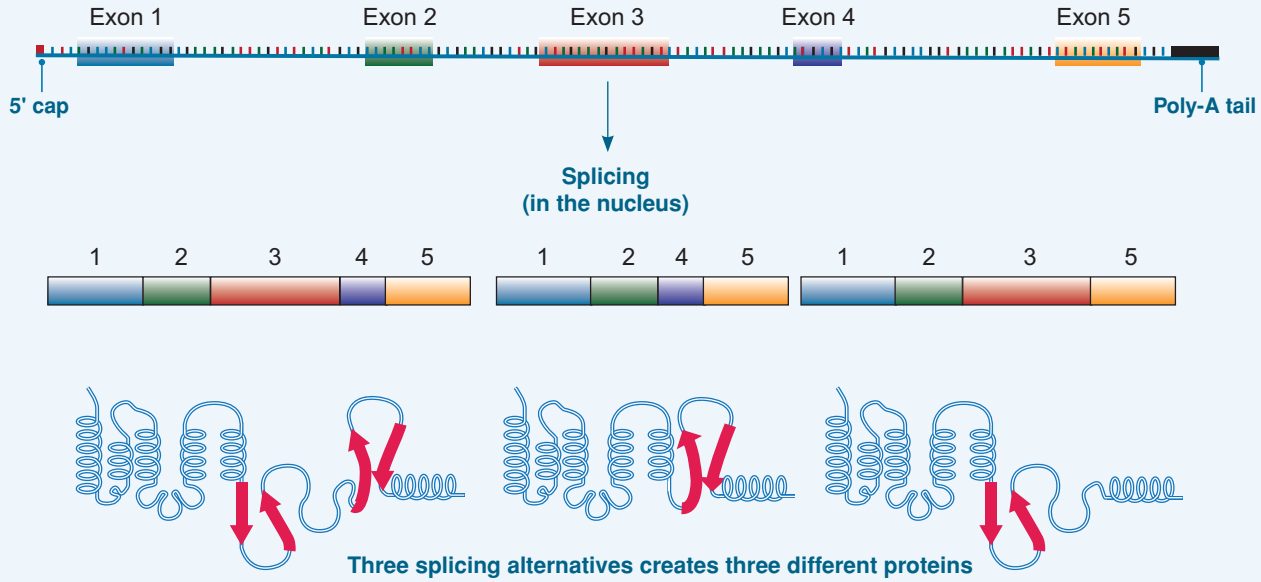
After transcription, the primary RNA transcript is modified by enzymes to create 'caps' and 'tails'. These modifications are part of the untranslated region (UTR) at each end of a gene. They stabilise the RNA, protect it from degradation, and help its transport through the nuclear pore. They are also important in translation although they are not translated themselves. The START and STOP points of translation are marked by darker green lines.

Modification after transcription

- As you have seen earlier, introns are removed from the primary mRNA transcript and the exons are spliced together. However, exons can be spliced together in different ways to create variations in the translated proteins. Exon splicing occurs in the nucleus, either during or immediately after transcription.
- In mammals, the most common method of alternative splicing involves exon skipping, in which not all exons are spliced into the final mRNA (below).

DID YOU KNOW?

Human DNA contains 25,000 genes, but produces up to 1 million different proteins. Modifications after transcription and translation allow several proteins to be produced from just one gene.



Three splicing alternatives creates three different proteins

1. What is the purpose of the caps and tail on mRNA? _____

_____

2. (a) What happens to the intronic sequences in DNA after transcription? _____

   (b) What is one possible fate for these introns? _____

3. How can so many proteins be produced from so few genes? _____

_____

_____

4. If a human produces 1 million proteins, but human DNA codes for only 25,000 genes, on average, how many proteins are produced per gene?

_____

ISBN: 978-1-98-856655-9

61 Translation

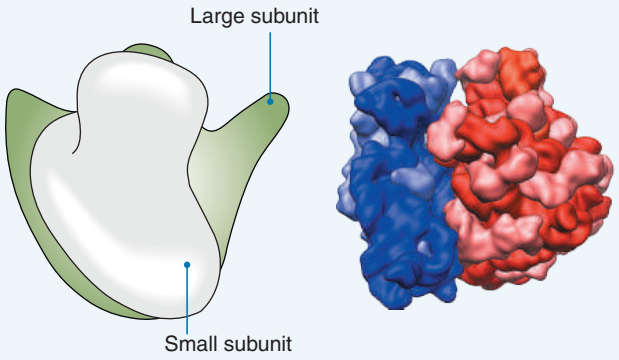
Key Idea: Translation is the final stage of gene expression in which ribosomes read the mRNA and decode (translate) it to synthesise a protein. This occurs in the cytoplasm.

In eukaryotes, translation occurs in the cytoplasm either at free ribosomes or ribosomes on the rough endoplasmic reticulum. Ribosomes translate the code carried in the mRNA molecules, providing a suitable environment for the linkage of amino acids delivered by transfer RNA (tRNA) molecules. Protein synthesis begins at the start codon and, as the ribosome wobbles along the mRNA strand, the polypeptide chain elongates. On reaching a stop codon, the ribosome subunits dissociate from the mRNA, releasing the protein.

Ribosome structure

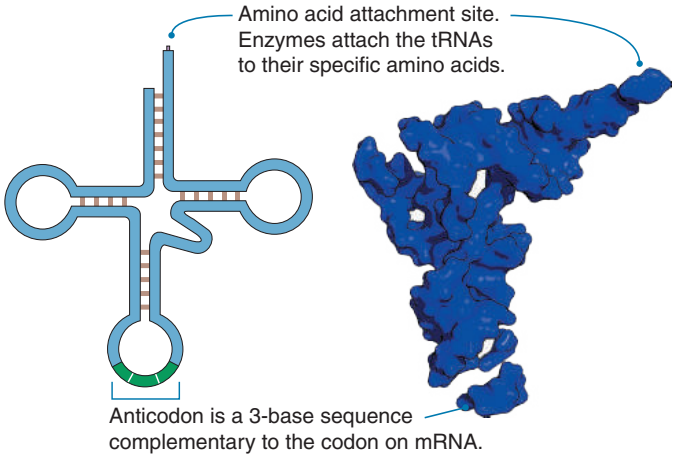
Ribosomes are made up of a complex of ribosomal RNA (rRNA) and ribosomal proteins. These small cellular structures direct the catalytic steps required for protein synthesis and have specific regions that accommodate transfer RNA (tRNA) molecules loaded with amino acids.

Ribosomes exist as two separate sub-units (below) until they are attracted to a binding site on the mRNA molecule, when they come together around the mRNA strand.



tRNA structure

tRNA molecules are RNA molecules, about 80 nucleotides long, which transfer amino acids to the ribosome as directed by the codons in the mRNA. Each tRNA has a 3-base anticodon, which is complementary to a mRNA codon. There is a different tRNA molecule for each possible codon and, because of the degeneracy of the genetic code, there may be up to six different tRNAs carrying the same amino acid.



1. Describe the structure of a ribosome: _____

_____

2. What is the role of each of the following components in translation?

   (a) Ribosome: _____

   (b) tRNA: _____

   (c) Amino acids: _____

   (d) Start codon: _____

   (e) Stop codon: _____

3. There are many different types of tRNA molecules, each with a different anticodon (HINT: see the mRNA table).

   (a) How many different tRNA types are there, each with a unique anticodon? _____

   (b) Explain your answer: _____

   _____

   _____

   (c) Determine the mRNA codons and the amino acid sequence for the following tRNA anticodons:

   tRNA anticodons: U A C U A G C C G C G A U U U

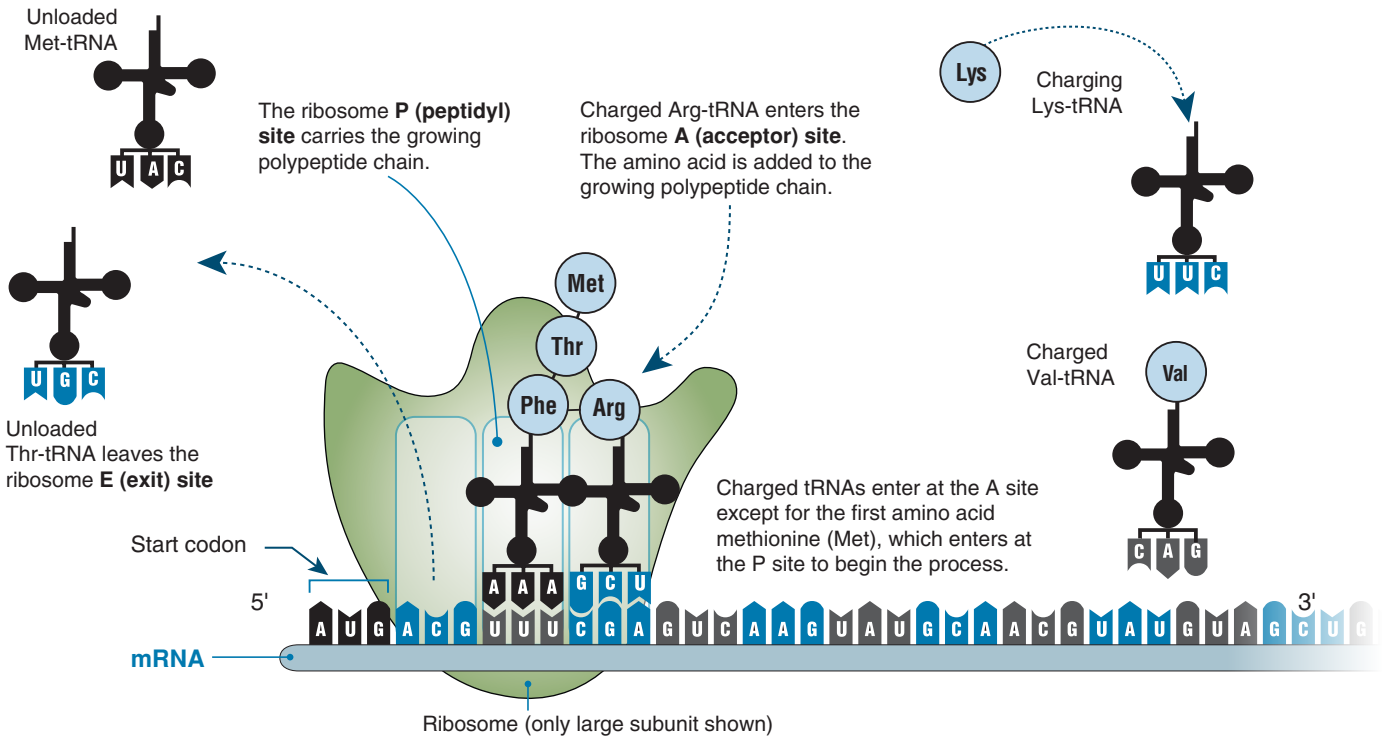
   Codons on the mRNA: _____

   Amino acids encoded: _____

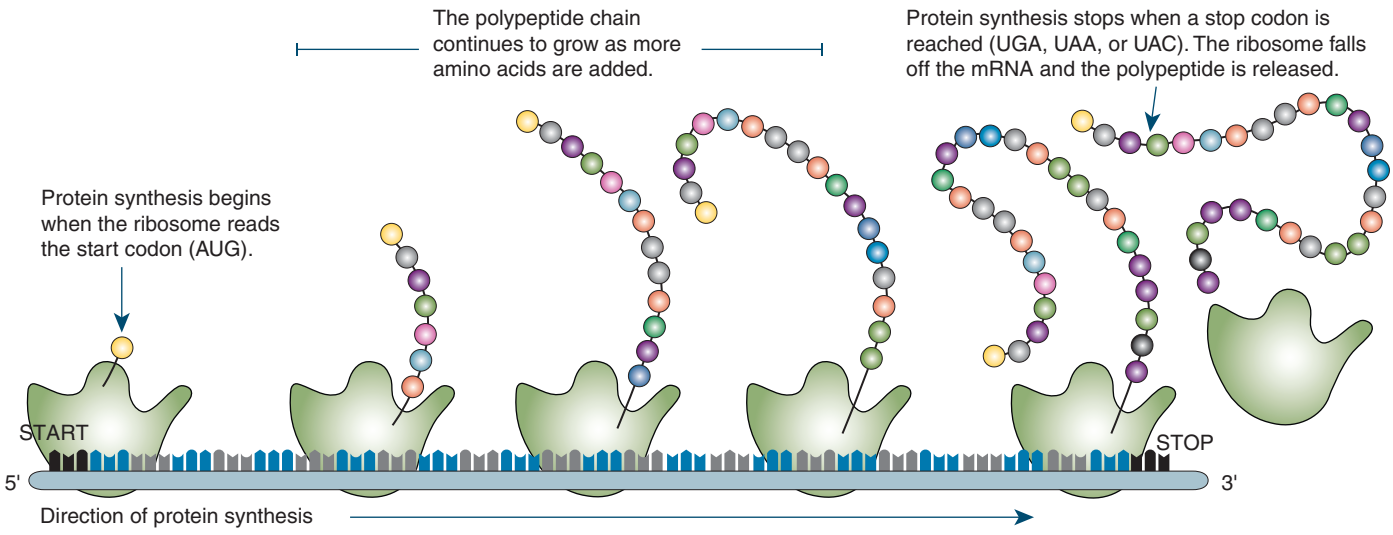
ISBN: 978-1-98-856655-9

tRNA molecules deliver amino acids to ribosomes

tRNA molecules match amino acids with the appropriate codon on mRNA. As defined by the genetic code, the anticodon specifies which amino acid the tRNA carries. The tRNA delivers its amino acid to the ribosome, where enzymes join the amino acids to form a polypeptide chain. During translation the ribosome 'wobbles' along the mRNA molecule joining amino acids together. Enzymes and energy are involved in charging the tRNA molecules (attaching them to their amino acid) and elongating the peptide chain.



The polypeptide chain grows as more amino acids are added



4. Describe the events occurring during translation: _____

_____

_____

_____

5. Many ribosomes can work on one strand of mRNA at a time (a polyribosome system). What would this achieve?

_____

_____

ISBN: 978-1-98-856655-9

62 Applications: Gene Expression Profiles

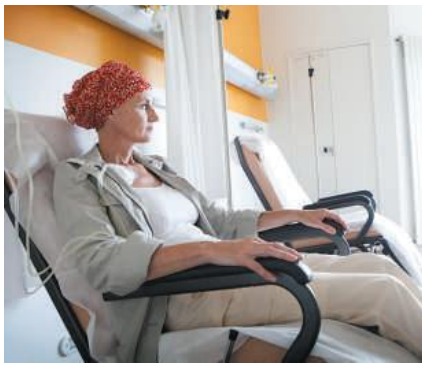
Key Idea: Identifying the mRNA present in cells can help identify, and lead to treatment of diseases such as cancer.

Every cell in an organism has the same DNA and therefore the same complement of genes. Different types of cells look and behave differently because they express different genes in different sequences for different lengths of time. Therefore, a skin cell expresses different genes and therefore different types of proteins, structures, and behaviours than a muscle cell. A cancerous cell expresses genes differently from a normal cell. Some genes will be expressed more than others and some will be expressed less. Gene expression profiling can identify these changes of expression.

Gene expression in cancer cells

- It is now possible to quantify the genes being expressed by looking for and measuring the different types and amounts of mRNAs and proteins found in a cell. This is called **gene expression profiling**. Using this technique, it is possible to identify the genes that are behaving incorrectly in cancer cells by comparing their gene expression profiles to the gene expression profiles of non-cancerous cells.
- The table (right) shows the top ten genes that are expressed more than normal (up regulated) and the top nine that are expressed less than normal (down regulated) in breast cancer patients. Most of the up regulated genes are involved in the cell cycle, whereas most of the down regulated genes are involved in lipid metabolism.

Over-expressed genes	Under-expressed genes
COL10A1	C7
MMP11	FABP4
GJB2	TIMP4
CST1	GPD1
KIAA1199	THRSP
MMP1	CIDEC
MMP13	ADH1B
CEACAM6	ADH1A
BUB1	KIAA1881
ASPM	

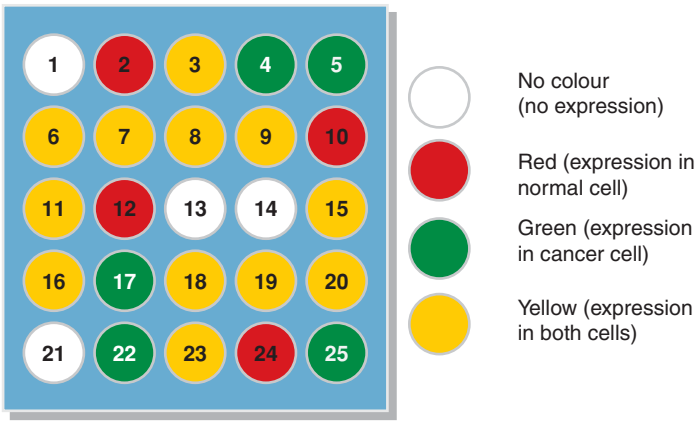


Using gene expression profiles

- Many drugs act on biochemical pathways in cells to alter some part of the cell's activity. Some people respond to these drugs better than other people, often because their cells have more of a crucial receptor. The receptor is produced by a biochemical pathway regulated by proteins, which are a product of gene expression. Variations in gene expression can determine the way a drug affects a person.
- Gene expression profiling can be used to identify suitable drugs for different patients. It can be especially useful in developing a chemotherapy regime for cancer treatment and identifying any side effects. This makes it possible to develop treatments that are more effective and more tolerable for the patient.

1. DNA chips (microarrays) can be used to determine which genes are being expressed in a cell. In one type of microarray, genes expressed in a normal cell turn the dot red while genes expressed in a cancer cell turn the dot green. Gene expression in both cells turns the dot yellow (the DNA chips are normally read by a laser).

   Each dot represents a different gene, so we can see which genes are being over or under expressed in a cancerous cell: green is over-expressed, red is under-expressed and yellow is expressed normally.



   (a) From the DNA chip above, which genes in the cell being tested are: over-expressed, under-expressed and being expressed normally?

   _____

   _____

   (b) Suggest how this information could be used to design medical treatment for a cancer patient:

   _____

   _____

   _____

2. Why would the over-expression of genes associated with the cell cycle be a major factor in causing cancer?

   _____

   _____

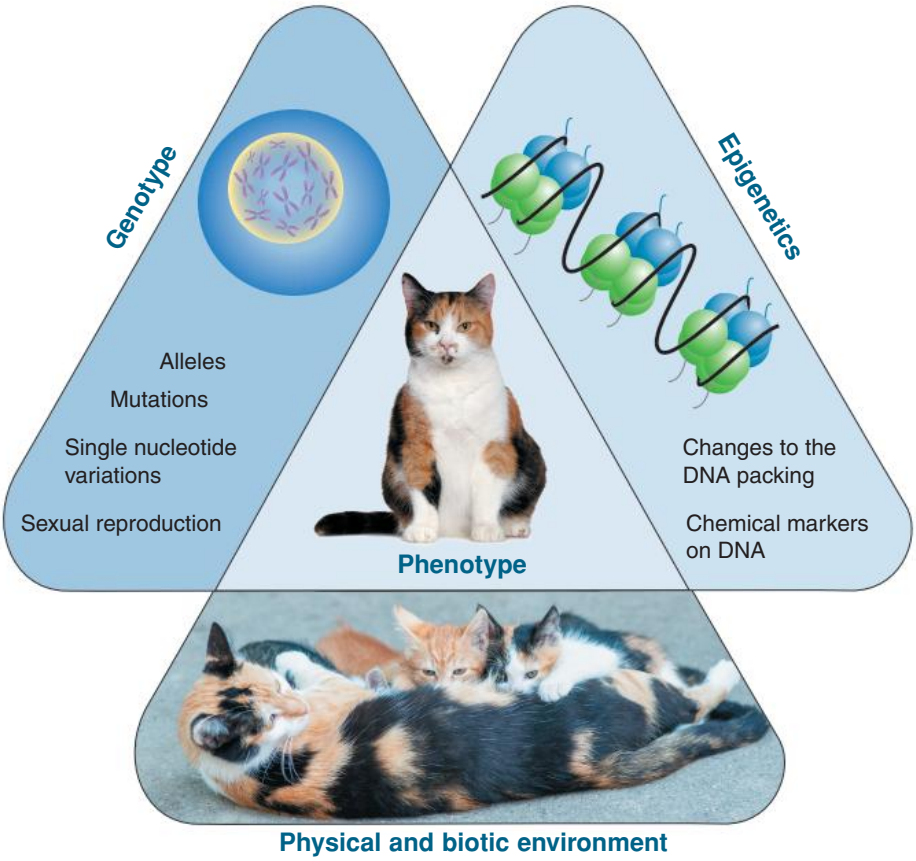
   _____

ISBN: 978-1-98-856655-9

63 Influences on Phenotype

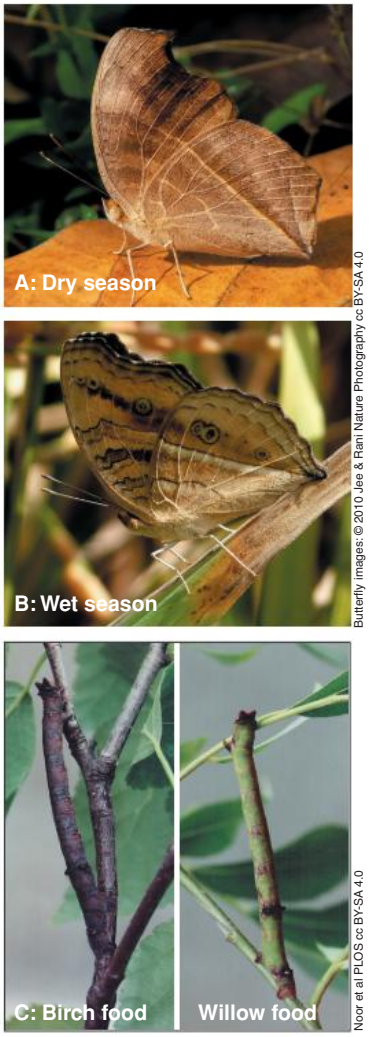
Key Idea: An organism's phenotype is influenced by the effects of the environment during and after development, even though the genotype remains unaffected.

An organism's phenotype is influenced by both its genes and the environment, and also the ways the genes are controlled, known as epigenetics. Even identical twins have minor differences in their appearance due to epigenetic and environmental factors such as diet and what the environment was like in the uterus during development. Genes, together with epigenetic and environmental factors, determine the unique phenotype that is produced.



- The phenotype is the product of the many complex interactions between the genotype, the environment, and the chemical tags and markers that regulate gene expression of the genes (epigenetic factors).
- **Polyphenism** is the expression of different phenotypes in organisms with the same genotype as a result of environmental or epigenetic influences. Examples include temperature dependent sex determination in reptiles, seasonal fur colour changes in Arctic foxes, seasonal changes in wing patterns in peacock pansy butterflies (photo panel A and B), and changes in body colour in response to food resources in peppered moth caterpillars (photo panel C).
- The amount of change in a phenotype due to environmental influences is called its phenotypic plasticity. Plants often have high phenotypic plasticity because they are unable to move and so must adjust to environmental changes throughout their lives.

Polyphenism: same genotype, different phenotype



Butterfly images: © 2010 Jee & Rani Nature Photography cc BY-SA 4.0

Noor et al PLOS cc BY-SA 4.0

See text left for explanation

1. (a) What are some sources of genetically induced variation? _____

   _____

   _____

   (b) What are some sources of environmentally induced variation? _____

   _____

2. Explain why genetically identical twins are not always phenotypically identical: _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

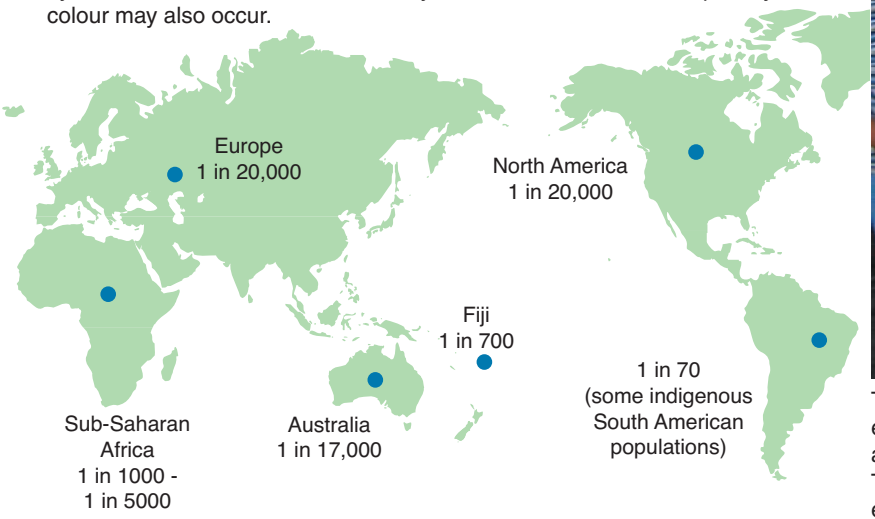
64 Genotype Influences Phenotype

Key Idea: Sexual reproduction, mutations, and variable regions of DNA (SNPs) each contribute to phenotype.

An individual's genotype is a result of many different factors. These include the combination of alleles they receive through sexual reproduction, changes in the DNA through mutation, and variability in DNA due to single nucleotide polymorphisms (SNPs – pronounced 'snips'). Each of these variations in genotype influence the phenotype of the individual.

Changes in phenotype: albinism

The photo (right) shows a family group where one of the children has an appearance quite different to the others. The child is an albino. Albinism is an inherited genetic disease resulting in the absence of pigmentation or colouration. There are several types of albinism. The most common, OCA, affects the skin, hair, and eyes. Albinos with OCA have white or extremely pale skin and hair, so must take care in the sun not to damage their skin and eyes. In humans with albinism, blue eyes are common, but red or pink eye colour may also occur.



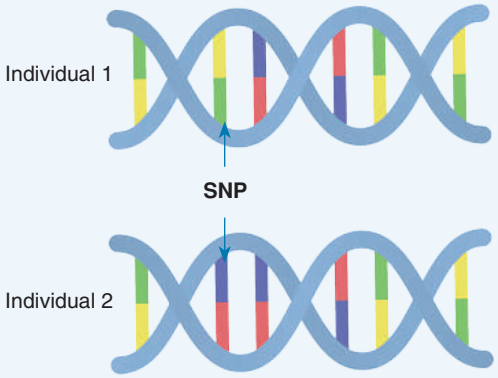
msdonna

The rate of OCA varies between countries. Some ethnic groups, and some populations in isolated areas show higher levels of albinism than others. This may be due to the presence of certain ethnic specific genetic factors called SNPs (gene variations in different parts of the world).

Factors contributing to genetic variation

How is it that some individuals have normal colouration and some are albinos? One factor contributing to the condition is genotype variation. This may arise through:

- **Sexual reproduction**: The processes of crossing over and independent assortment during meiosis increases genetic variability in the gametes (egg and sperm). Random fertilisation between egg and sperm further enhances genetic variation in the resulting zygote.
- **Alleles**: Alleles are different versions of a gene. Different alleles result from changes in the DNA sequence. This increases genetic variation between individuals.
- **Mutation**: Mutations are changes to a DNA sequence and are a source of new alleles. Mutations come from errors in DNA copying or can be caused by external factors called mutagens, e.g. UV radiation. Mutations may change only a single base pair in the DNA, or large parts of chromosomes.
- **Single nucleotide polymorphism (SNPs)**: SNPs (pronounced snips) are a single change in a nucleotide base between individuals (diagram, top right). SNPs are the most common form of variation in the human genome. It is estimated that the human genome contains 3–10 million SNPs. Some SNPs are associated with different variants of albinism.



DID YOU KNOW?

What's the difference between a mutation and SNP?

SNPs and single nucleotide substitution mutations are very similar. To be classified as a SNP, the substitution must occur in more than 1% of the population.

1. What health problem might people with albinism suffer from because of low pigmentation levels? _____

_____

_____

ISBN: 978-1-98-856655-9

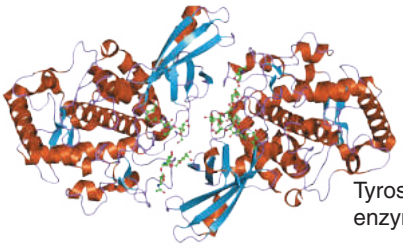
The genome and albinism

Allele combination

We can represent the alleles for albinism as **P** (normal pigmentation) and **p** (albino pigmentation) The abnormal allele (**p**) for OCA is inherited in a homozygous recessive pattern. An individual must have two copies of the abnormal gene to be albino. Individuals with one copy are carriers; they show no characteristics of being albino, but they can pass the gene for albinism on to their offspring.

SNPs

Most SNPs do not occur in regions coding for genes, but when they do they can be associated with disease. Sometimes SNPs can occur more often in certain populations (see previous page) so can be useful for tracking disease genes. Albino kangaroos and wallabies are fairly uncommon and SNP analysis is limited. Only one albino is born for every 50,000-100,000 births. The rate of albinism is much higher in humans (on average one in every 17,000 - 20,000 births).


Tyrosinase enzyme

Sexual reproduction

The law of independent assortment (sorting chromosomes during meiosis) means there is no way to predict which gametes will receive an albino allele. However, the probability of producing albino offspring can be predicted by studying the inheritance pattern for the disease. You will study inheritance patterns in the next chapter.

Mutation

Several gene mutations are known to result in albinism. The most common form of OCA is caused by a mutation to the TYR gene. The TYR gene produces an enzyme called tyrosinase, which is needed to make the pigment melanin. Melanin gives the skin, hair, and eyes their colour. Without tyrosinase there is no melanin, which is why albinos have a pale or white appearance.

2. Explain the difference between a single nucleotide mutation and a SNP: _____

3. Using the alleles P (normal pigmentation) and p (albino pigmentation), write the allele combination for someone who:

   (a) Has albinism: _____

   (b) Is a carrier of albinism: _____

   (c) Does not have albinism and is not a carrier: _____

4. Why do carriers for albinism not show symptoms of albinism? _____

5. Explain how the TYR gene mutation is involved in producing the classic OCA phenotype: _____

ISBN: 978-1-98-856655-9

65 Environment and Phenotype

Key Idea: The environment can play a big part in an organism's eventual phenotype.

Environmental factors, including physical factors such as temperature, and biotic factors such as presence of predators, can influence how genes are expressed. Factors such as heat or chemicals can turn genes on (genes are expressed) or off. When, and for how long, the genes are expressed can have large effects on an organism's eventual phenotype.

The effect of temperature

Crocodiles hatching

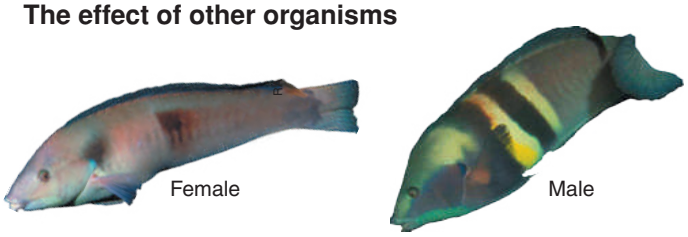
The sex of turtles, crocodiles, and the American alligator is determined by the incubation temperature during embryonic development. In some species, high incubation temperatures produce males and low temperatures produce females. In other species, the opposite is true. Temperature regulated sex determination may provide an advantage by preventing inbreeding.

Cindy McCravey cc2.0

Colour pointing

Siamese kittens are born fully white, but develop dark patches after birth. The colour is caused by the pigment melanin. In Siamese cats, the tyrosinase enzyme involved in melanin production is mutated and inactivated at normal body temperature. Tyrosinase can only function to produce melanin in cooler areas of the body (ears, feet, tail). In the rest of the body where the temperature is higher, no melanin is produced.

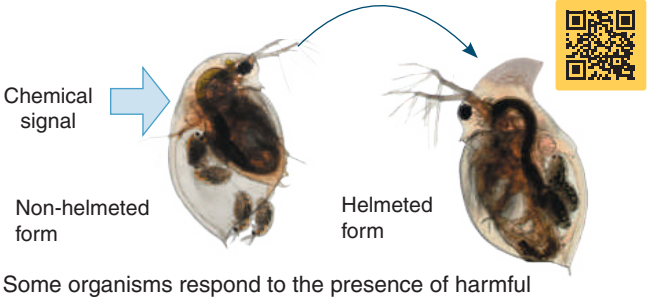
The effect of other organisms



Female

Male

For some fish species, such as *Coris sandageri* (above), the presence of other individuals of the same species may control sex determination. The fish live in groups consisting of a single male with attendant females and juveniles. In the presence of a male, all juveniles become females. When the male dies, the dominant female will undergo changes in physiology and appearance to become a male.



Some organisms respond to the presence of harmful organisms by changing their morphology or body shape. When the water flea *Daphnia* is exposed to predatory phantom midge larvae it develops a helmet and/or tail spine which make it more difficult to attack. They also produce young with the same defensive structures. These responses are generated in response to chemicals produced by the predator.

1. Why are the darker patches of fur in Siamese cats only present on the face, paws, and tail? _____

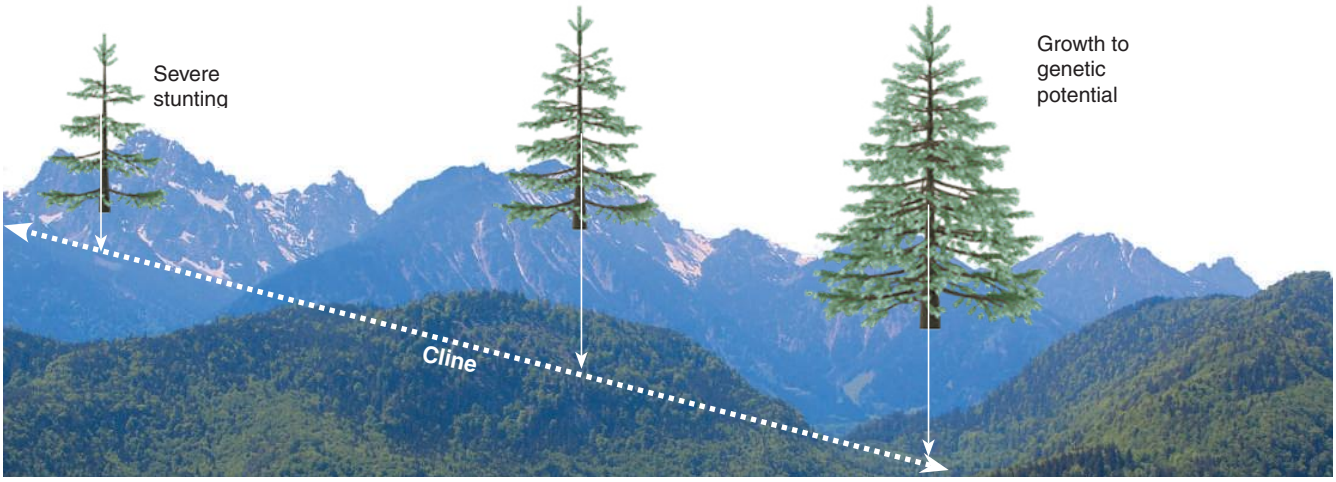
2. (a) How is helmet and spine development in *Daphnia* a response to environment? _____

   (b) How does the phenotypic response help the animal survive? _____

ISBN: 978-1-98-856655-9

Environment can determine whether or not an organism reaches its genetic potential

Increasing altitude can stunt the phenotype of plants with the same genotype. In many tree species, such as spruce (below) and mountain beech, plants at low altitude grow to their full genetic potential, but growth becomes more and more stunted as elevation increases and abiotic factors, such as temperature, change. Growth is gnarled and plants are shorter at the highest sites. Gradual change in phenotype over an environmental gradient is called a cline.



How do chemicals affect hydrangea flowers?

- Changes in the chemical environment influence flower colour in hydrangeas (right). They have blue flowers when they are grown in acidic soil (pH <7.0) and pink flowers when grown in neutral to basic soils (≥ 7.0).
- The colour change is a result of the mobility and availability of aluminum ions (Al^{3+}) at different pH levels.
- At low pH Al^{3+} is highly mobile. It binds with other ions and is taken up into the plant, reacting with the usually red/pink pigment in the flowers to form a blue colour.
- In soil pH at or above 7.0, the aluminum ions combine with hydroxide ions to form insoluble and immobile aluminum hydroxide ($Al(OH)_3$). The plant doesn't take up the aluminum and remains red/pink.
- Other conditions such as high phosphorus levels can also affect aluminum mobility and availability.

Soil pH <7.0: blue flowers

Soil pH > 7.0: pink flowers

3. Describe an example to illustrate how genotype and environment contribute to phenotype: _____

4. (a) What is a cline? _____

(b) Describe how the phenotype of the spruce changes with an increase in altitude: _____

(c) Physical (abiotic) factors change with altitude. Suggest what physical factors may influence the tree's phenotype:

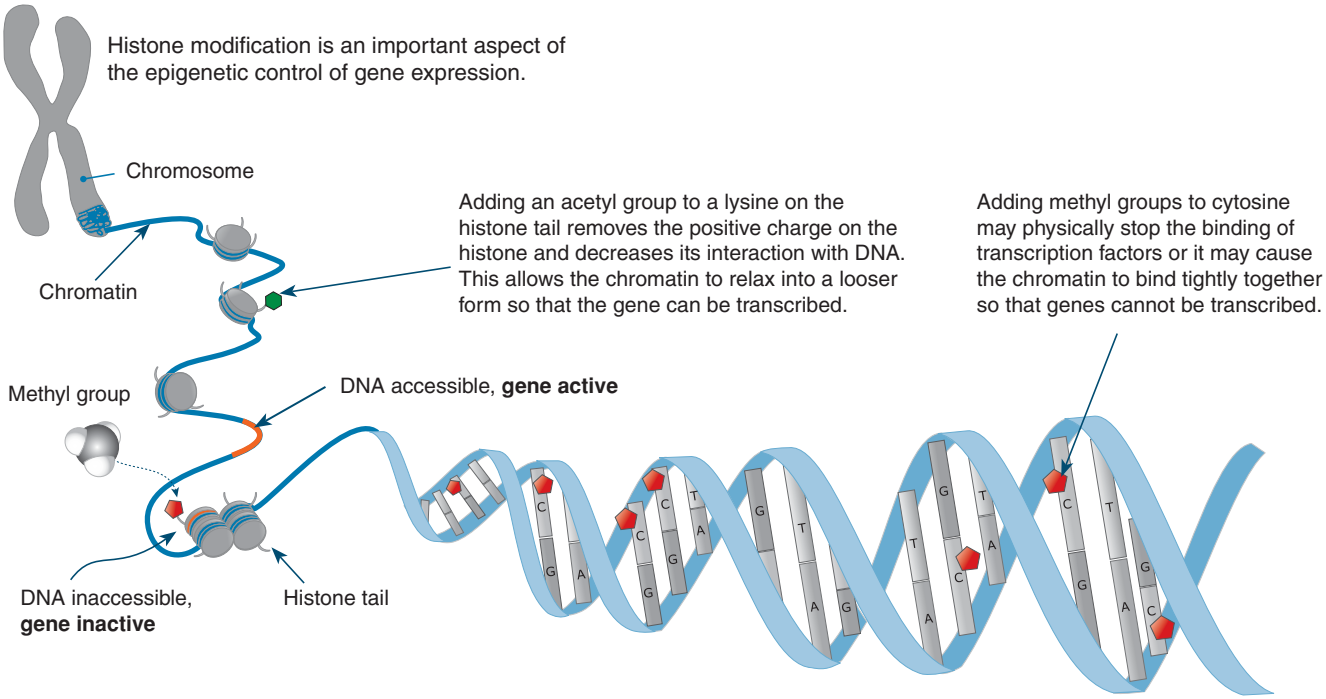
ISBN: 978-1-98-856655-9

66 Epigenetic Regulation of Gene Expression

Key Idea: The mechanisms by which the environment modifies the expression of genes are often epigenetic.

As you saw earlier, gene expression can be influenced in part by the environment. But how is the influence of environment moderated? Sometimes, as with colour pointing in cats, the environment directly influences a protein's function. Most often though, the regulation is epigenetic. Epi- means 'on top of' or 'extra to'. Epigenetic factors are external to the gene itself, e.g. chemical tags, that influence how that gene is expressed. Epigenetic regulation is achieved by modifying the way the DNA is packaged and its availability to be transcribed. The DNA sequence is unchanged.

The regulation of gene expression in eukaryotes is a complex process beginning before the DNA is even transcribed. The packaging of DNA regulates gene expression either by making the nucleosomes in the chromatin pack together tightly (**heterochromatin**) or more loosely (**euchromatin**). This affects whether or not RNA polymerase can attach to the DNA and transcribe it.



1. What is epigenetics? _____

_____

_____

2. (a) Describe the effect of histone modification and adding methyl groups on DNA packaging: _____

_____

_____

(b) How do these processes affect transcription of the DNA? _____

_____

_____

3. The graphs (right) show the relative amount of genomic methylation and the effect of this on the frequency of gene expression. Describe the relationship between methylation and gene expression:

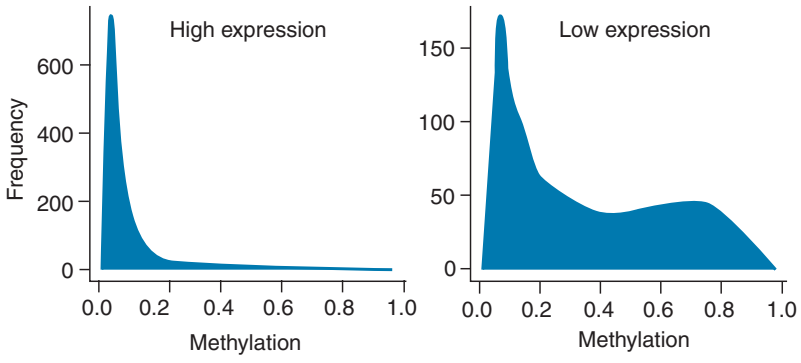
_____

_____

_____

_____

_____



ISBN: 978-1-98-856655-9

Epigenetics affects development

- One of the biggest debates in the study of development is the idea of nature versus nurture. To what extent do genes or the environment affect development and phenotype?
- A 2004 study of the grooming of rat pups by their mothers helps provide some insight. In this study, the quality of care by a pup's mother affected how the pup behaved when it reached adulthood.
- Rat pups that were groomed more often by their mother were better at coping with stress than pups that received less grooming. What's more, it was shown that the effect was caused by changes in the expression of the glucocorticoid receptor, which plays a role in the response to stress.
- DNA analysis found differences in the way the DNA was chemically tagged. Rats that received a lot of grooming had DNA that allowed for greater transcription and so had higher expression of the glucocorticoid receptor. The opposite was true for rats that received little grooming.

Twins in space

- Twin studies can provide a lot of information about how the environment affects gene expression. The studies are often done when identical twins have been separated at birth (usually because one or both of them are adopted out). Their similarities and differences can then be studied to assess how much the environment influenced their development.
- In 2015, NASA astronaut Scott Kelly blasted into space for a year long stay on the International Space Station. His identical twin brother Mark remained on Earth. This gave NASA a chance to study the real effects of space travel on the human body. Importantly, the gene expression of the men could be measured before and after Scott went to space.
- It was found that six months after Scott's return, 7% of his genes had not returned to their normal level of gene expression. Also, although there was no decrease in Scott's cognitive abilities, there was a decrease in his speed and accuracy until his readjustment to Earth gravity. The space environment had altered Scott Kelly's gene expression compared to his identical twin Mark Kelly.

NASA

4. (a) Describe how grooming of their pups by mother rats affected the pups in the long term: _____

_____

(b) How could this have been achieved by epigenetics? _____

_____

_____

5. How might twin studies help the study of gene-environment responses? _____

_____

_____

_____

6. What evidence is there that epigenetics can have long term to permanent effects on gene expression? _____

_____

_____

_____

_____

7. When a zygote forms at fertilisation, most of the epigenetic tags are erased so that cells return to a genetic 'blank slate', ready for development to begin. However, some epigenetic tags are retained and inherited. Why do you think it might be advantageous to inherit some epigenetic tags from a parent?

_____

_____

_____

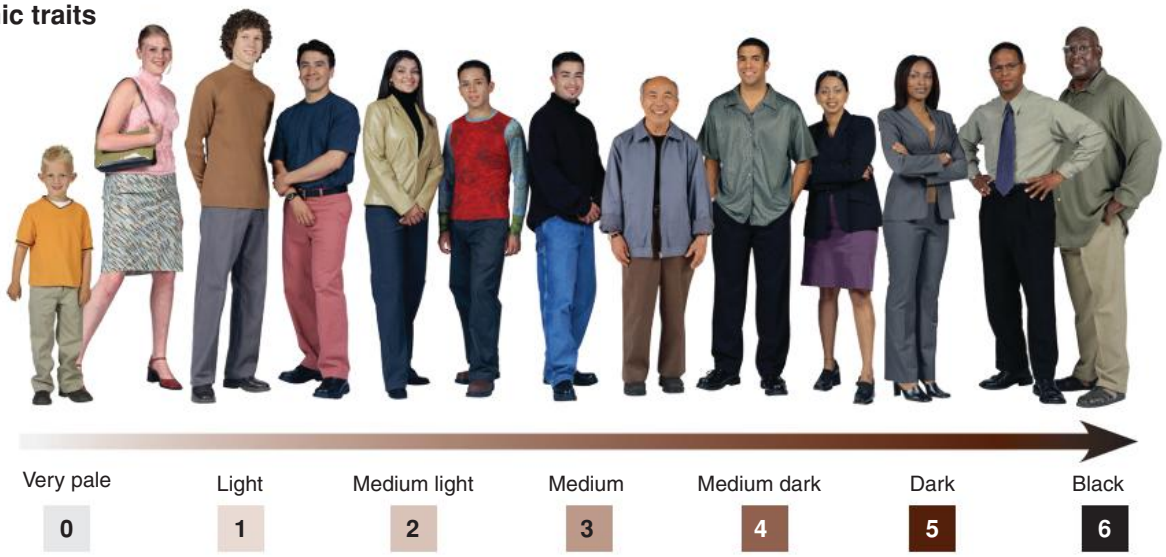
ISBN: 978-1-98-856655-9

67 Genes, Environment, and Continuous Variation

Key Idea: Many phenotypes are affected by multiple genes.

Many phenotypes are controlled by more than one gene. This is called polygeny or polygenic inheritance. As phenotype is controlled by many genes and alleles, a large range is possible. Combined with environmental effects, this produces **continuous variation** within the population. Two examples in humans are skin colour and height.

Polygenic traits



Polygenic traits are usually identified by:

- Traits are usually quantified by measuring rather than counting.
- Two or more genes contribute to the phenotype.
- Phenotypic expression is over a wide range (often in a bell shaped curve).
- Polygenic phenotypes include skin colour, height, eye colour, and weight.

*Multiple genes (many **genes** contributing to a phenotype) are quite different to multiple alleles (many **alleles** present in the population for one phenotypic characteristic).*

It is estimated that skin colour is controlled by at least eight genes (probably more). There are various ways to compare skin colour. One is shown above, in which there are seven shades ranging from very pale to very dark. Most individuals are somewhat intermediate in skin colour.

The table (right) shows a cross between three genes involved in skin colour, A, B, and C, each with two alleles (AaBbCc x AaBbCc). This is sufficient to give the seven shades of skin colour shown above. The shaded boxes indicate their effect on skin colour when combined. No dominant allele results in a lack of dark pigment (aabbcc). Full pigmentation (black) requires six dominant alleles (AABBCC). Note that for three genes with two alleles each there are 23 x 23 = 8 x 8 = 64 possible genotypes.

Gametes	ABC	ABc	AbC	Abc	aBC	aBc	abC	abc
ABC	AABB CC	AABB Cc	AABb CC	AABb Cc	AaBB CC	AaBB Cc	AaBb CC	AaBb Cc
ABc	AABB Cc	AABB cc	AABb Cc	AABb cc	AaBB Cc	AaBB cc	AaBb Cc	AaBb cc
AbC	AABb CC	AABb Cc	AAbb CC	AAbb Cc	AaBb CC	AaBb Cc	Aabb Cc	Aabb Cc
Abc	AABb Cc	AABb cc	AAbb Cc	AAbb cc	AaBb Cc	AaBb cc	Aabb Cc	Aabb cc
aBC	AaBB CC	AaBB Cc	AaBb CC	AaBb Cc	aaBB CC	aaBB Cc	aaBb CC	aaBb Cc
aBc	AaBB Cc	AaBB cc	AaBb Cc	AaBb cc	aaBB Cc	aaBB cc	aaBb Cc	aaBb cc
abC	AaBb CC	AaBb Cc	Aabb CC	Aabb Cc	aaBb CC	aaBb Cc	aabb CC	aabb Cc
abc	AaBb Cc	AaBb cc	Aabb Cc	Aabb cc	aaBb Cc	aaBb cc	aabb Cc	aabb cc

1. (a) What is polygeny? _____

_____

(b) How does polygeny contribute to continuous variation? _____

_____

_____

2. Study the cross between the A, B, and C genes above. Write down the frequencies of the seven phenotypes (0-6):

_____

_____

ISBN: 978-1-98-856655-9

Investigation 3.1 Measuring continuous variation

See appendix for equipment list.

1. Choose one variable which occurs as a result of continuous variation (e.g. height, weight, hand span, or foot length) and write the variable you will be investigating here:

   _____

2. Select 30–50 classmates to be your sample. Measure the variable of interest (to one decimal place) and record the results in the space for raw data below.
3. Decide on appropriate frequency for the data, then record it as a tally chart in the space below.

Raw data

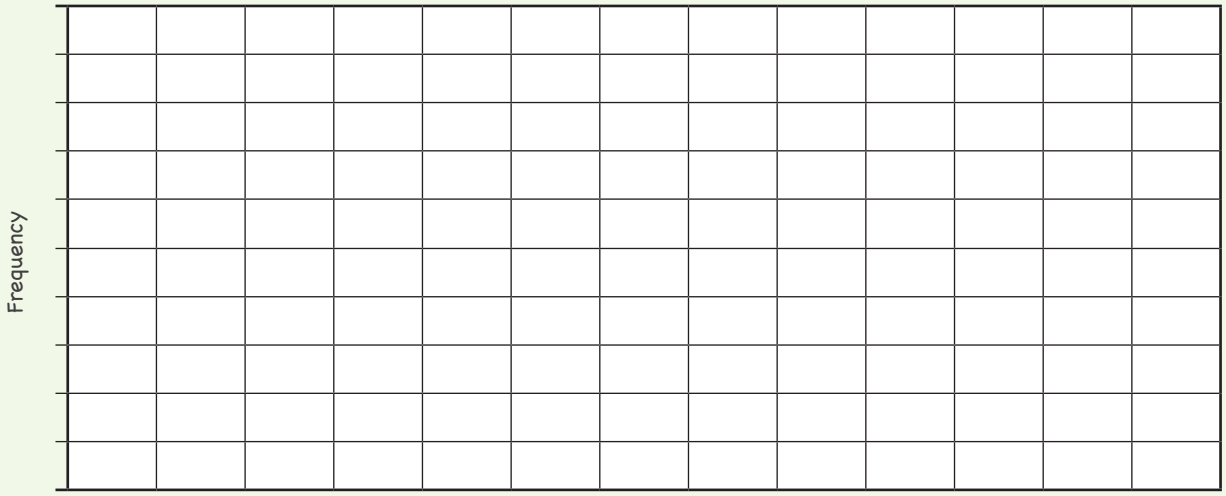
Tally chart

4. Summarise the features of your data in the table (right). You can enter your data on a spreadsheet to make it easier to calculate if you want.
5. Plot the tally chart data as a histogram on the grid (next page).

Number of entries	
Sum	
Mean	
Standard deviation	

ISBN: 978-1-98-856655-9

Variable: .....



3. (a) Describe the pattern of the distribution shown in your graph: _____

(b) What is the genetic basis of this distribution? Give a brief explanation of what this means: _____

(c) What is the importance of a large sample size when gathering data relating to a continuous variable?_____

4. Explain the differences between continuous and discontinuous variation, giving examples to illustrate your answer:

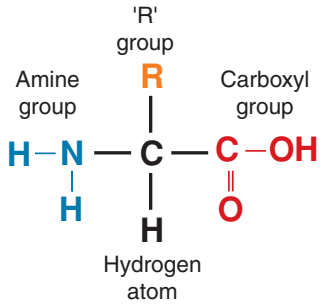
ISBN: 978-1-98-856655-9

68 Amino Acids Make up Proteins

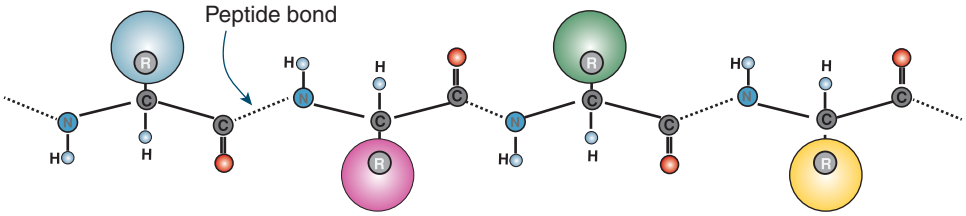
Key Idea: Amino acids join together in a linear chain by condensation reactions to form polypeptides. The sequence of amino acids in a protein is defined by a gene and encoded in the genetic code. Polypeptides are broken apart into amino acids by hydrolysis (the opposite reaction to condensation).

Amino acids are the basic units from which proteins are made. Twenty amino acids commonly occur in proteins and they can be linked in many different ways by peptide bonds to form a huge variety of polypeptides. Proteins are made up of one or more polypeptide molecules.

The structure and properties of amino acids

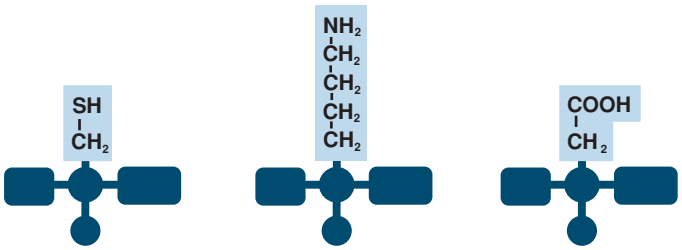


- Amino acids are the building blocks of proteins. They are linked by peptide bonds (below and opposite) to form long chains called polypeptides, which are the basis of proteins. All amino acids have a common structure (left) with an amine group (blue), a carboxyl group (red), a hydrogen atom, and a functional or 'R' group (orange).
- Each type of amino acid has a different functional R group (side chain). Each functional R group has a different chemical property.
- Amino acids are represented by a single upper case letter or a three-letter abbreviation. For example, proline is known by the letter P or the three-letter symbol Pro.



Different amino acids have different R groups

- The R group in the amino acid determines the chemical properties of the amino acid. Different amino acids have different R groups and therefore different chemical properties. Amino acids can be grouped according to these properties. Common groupings are nonpolar (hydrophobic), polar (hydrophilic), positively charged (basic), or negatively charged (acidic).
- The property of the R group determines how the amino acid interacts with others and how the amino acid chain will fold up into a functional protein. For example, the hydrophobic R groups of soluble proteins will be folded into the protein's interior.



Cysteine
The 'R' group of cysteine forms **disulfide bridges** with other cysteines to create cross linkages in a polypeptide chain.

Lysine
The 'R' group of lysine gives the amino acid an **alkaline** property.

Aspartic acid
The 'R' group of aspartic acid gives the amino acid an **acidic** property.

1. What makes each of the amino acids in proteins unique, and how does this uniqueness contribute to protein structure?

2. Do some research to assign each of the 20 amino acids found in proteins to one of the four groups below. Use a standard 3-letter code to identify each amino acid:

   (a) Nonpolar (hydrophobic): _____

   (b) Polar (hydrophilic): _____

   (c) Positively charged (basic): _____

   (d) Negatively charged (acidic): _____

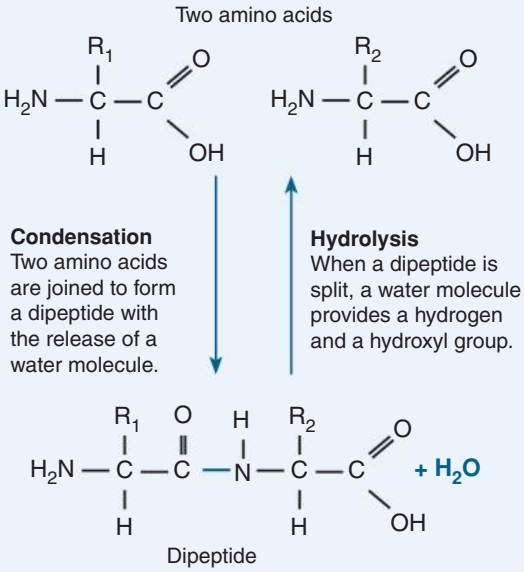
3. (a) Which type(s) of amino acids would you find on the surface of a soluble protein? Which type(s) would you find in the interior? Explain:

   (b) What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

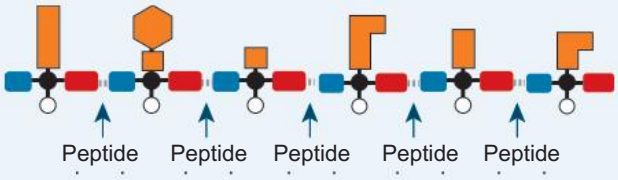
ISBN: 978-1-98-856655-9

Polypeptides are made by condensation and broken down by hydrolysis

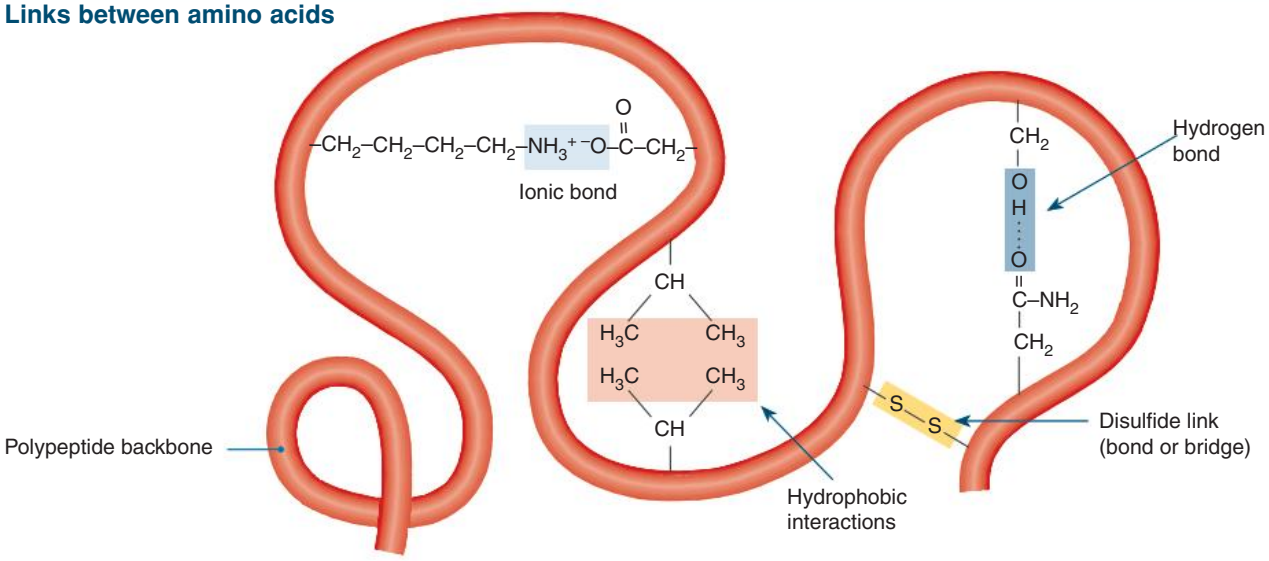
- Amino acids are linked by **peptide bonds** to form **polypeptide chains** of up to several thousand amino acids.
- Peptide bonds form between the carboxyl group of one amino acid and the amine group of another (right). Water is formed as a result of this bond formation so the reaction is called a condensation.
- The sequence of amino acids in a polypeptide is called the **primary structure** and is determined by the order of nucleotides in DNA and mRNA.



Polypeptide chain



Links between amino acids



4. (a) What type of bond joins neighbouring amino acids together? _____

   (b) How is this bond formed? _____

   (c) Circle this bond in the dipeptide above:

   (d) How are di- and polypeptides broken down? _____

5. Use the diagram above to answer the following:

   (a) Name the different interactions that can shape the polypeptide: _____

   _____

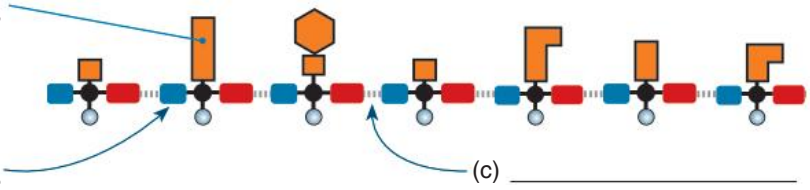
   (b) Which of the interactions would be the strongest? _____

6. In the diagram below, identify the following, *R group, amine group, peptide bond*:

   (a) _____

   (b) _____

   (c) _____



ISBN: 978-1-98-856655-9

69 Separating Amino Acids by Chromatography

Key Idea: Amino acids can be separated and identified using chromatography.

There are twenty essential amino acids used by the body to make proteins. Because each amino acid has a different chemical size and shape, they can be separated using thin layer chromatography. In thin layer chromatography, the mobile phase is the solvent which will separate the molecules. The stationary phase is a thin layer of adsorbent material (e.g. silica gel or cellulose) attached to a solid plate. A sample is placed near the bottom of the plate which is placed in an appropriate solvent (the mobile phase).

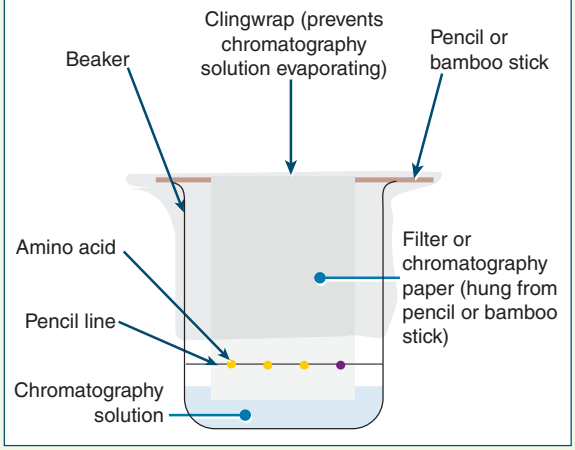
Investigation 3.2 Separating amino acids

See appendix for equipment list.

Do not handle the chromatography to avoid contaminating it. Solvents and ninhydrine solution should be used in a fume hood. You should wear protective eyewear and gloves.

1. Wear safety gloves and goggles during this investigation.
2. Cut a piece of filter paper or chromatography paper into a strip 5–6 cm wide. It should be long enough to reach from the top of a beaker to the bottom and not so wide that it touches the sides.
3. Use a pencil to draw a line across the width of the paper 1 cm from the bottom to mark the start position. Lightly mark 4 equally spaced dots along the starting line. Lightly number the dots.
4. Use a toothpick to place a drop of one of the 1% amino acid solutions (leucine, lysine, or glycine) to the first dot, the second amino acid on the second dot and so on. Place the unknown solution on the fourth dot. Record which amino acid was placed on which dot.
5. In a fume hood, pour the solvent solution into a beaker to a depth of just over 1 cm. Set up the chromatography paper as in the diagram on the right.
6. Cover the solution with parafilm or clingwrap and leave for up to an hour or until the solvent front is about 1 cm from the top of the chromatography paper.

Chromatography set up



7. In a fume hood, remove the paper and mark the solvent front with a pencil. Dry with a hair dryer. Pour the solvent into the waste container provided by your teacher.
8. In a fume hood, spray the chromatography paper with ninhydrin solution and dry with a hair dryer on heat for about 5 minutes. The spots of amino acid should become visible. Alternatively, the positions of the amino acids can be viewed with a black light.
9. Identify the unknown amino acid. Measure the distance from the start position to each amino acid and record. Measure the distance from the start position to the solvent front and record.

1. What was the unknown amino acid? _____

2. Use the formula below to calculate the R_f values for the amino acids you used. Each amino acid has its own Rf value. Rf values can be used to identify unknowns in reaction solutions.

$$R_f = \frac{\text{Distance travelled by spot (from start position)}}{\text{Distance travelled by solvent (from start position)}}$$

ISBN: 978-1-98-856655-9

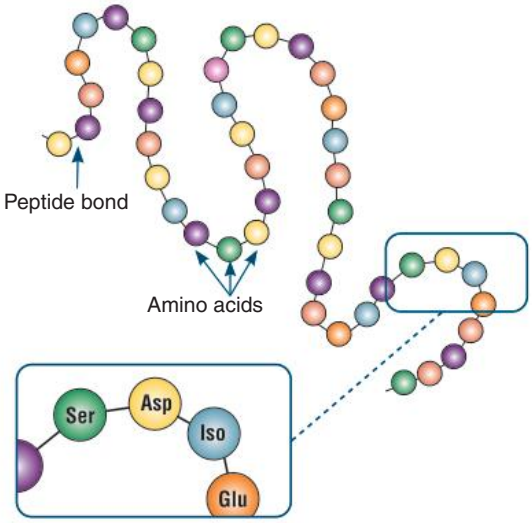
70 Protein Structure is Hierarchical

Key Idea: The three-dimensional shape and function of a protein is determined by the sequence and type of amino acid.

Proteins are large, complex **macromolecules**, built up from repeating units called **amino acids**. Proteins account for more than 50% of the dry weight of most cells and are important in virtually every cellular process. The various properties of the amino acids, influenced by the R groups, determine how the polypeptide chain folds up. This three dimensional **tertiary structure** gives a protein its specific chemical properties. If a protein loses this precise structure through **denaturation**, it is usually unable to carry out its biological function.

Primary (1°) structure

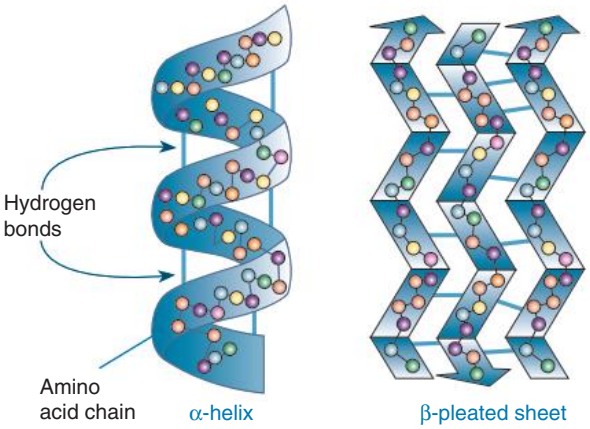
The amino acid sequence



Hundreds of amino acids are linked by peptide bonds to form polypeptide chains. The attractive and repulsive charges on the amino acids determine the higher levels of organisation in the protein and its biological function.

Secondary (2°) structure

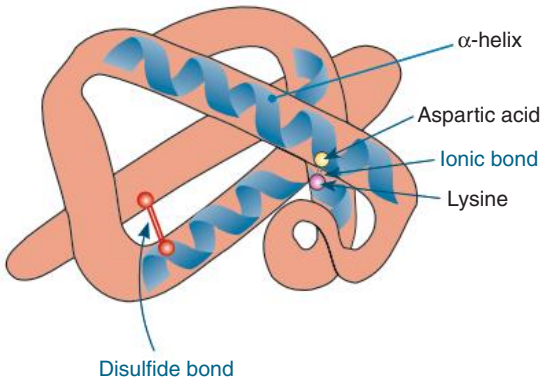
α-helices or β pleated sheets



Secondary (2°) structure is maintained by hydrogen bonds between neighbouring CO and NH groups. Hydrogen bonds are individually weak but collectively strong and determine how the primary chain folds. The coiled α-helix and β-pleated sheet are common 2° structures. Most globular proteins contain regions of both 2° configurations.

Tertiary (3°) structure

Folding of the 2° structure

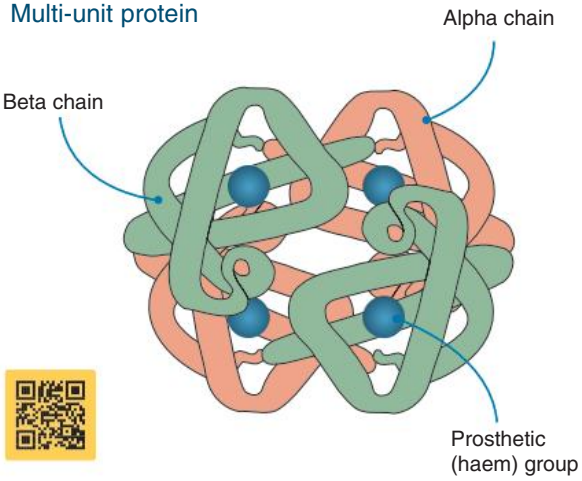


The tertiary (3°) structure is maintained by more distant interactions such as **disulfide bridges** between cysteine amino acids, ionic bonds, and hydrophobic interactions.

A protein's 3° structure is the three-dimensional shape formed when the 2° structure folds up and more distant parts of the polypeptide chains interact.

Quaternary (4°) structure

Multi-unit protein



Some complex proteins are only functional when existing as a group of polypeptide chains. Haemoglobin (above) has a 4° structure made up of two alpha and two beta polypeptide chains, each enclosing a complex iron-containing prosthetic (or haem) group.

A protein's 4° structure describes the arrangement and position of each of the subunits in a multiunit protein. The shape is maintained by the same sorts of interactions as those involved in 3° structure.

ISBN: 978-1-98-856655-9

1. Describe the main features in the formation of each part of a protein's structure:

   (a) Primary structure: _____

   _____

   (b) Secondary structure: _____

   _____

   (c) Tertiary structure: _____

   _____

   (d) Quaternary structure: _____

   _____

2. How are proteins built up into a functional structure? _____

   _____

   _____

   _____

3. Strong chemicals and extremes of temperature or pH can disrupt the bonds in proteins. What would this do to the protein's function and why?

   _____

   _____

   _____

Investigation 3.3 Modelling protein structure

See appendix for equipment list.

Work in pairs for this activity.

1. You will need pipe cleaners with four colours. We have used 2 white, 2 pink, 2 purple, and 4 blue but you can swap out for the colours you have. Each colour represents a different amino acid.
2. Twist a loop in the centre of each pipe cleaner (Figure 1). The twist represents the amino acid's functional group.
3. Join the amino acids together (figure 2) by twisting their arms together in the following sequence:
   1) white 2) pink 3) blue 4) purple 5) blue 6) pink 7) blue 8) white 9) blue 10) purple.

   What level of protein organisation does the structure in Figure 2 represent?

   _____
4. Attach sticky tape to the loops of the purple pipe cleaners and to one arm of each of the blue pipe cleaners. These represent places where hydrogen bonding can occur.

Figure 1

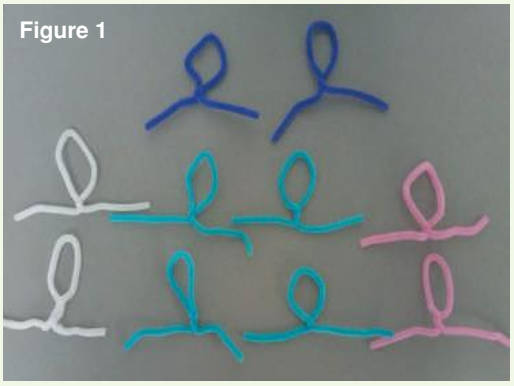
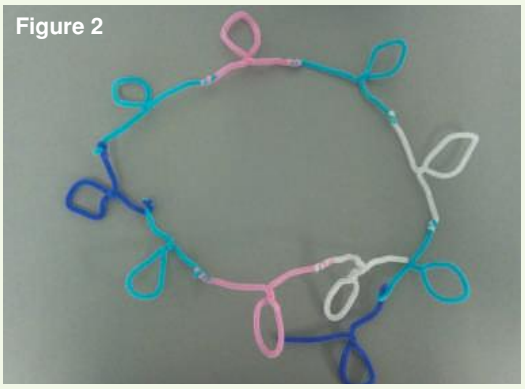


Figure 2



ISBN: 978-1-98-856655-9

5. Join the sticky tape together between amino acids 3 and 5 and also between amino acids 7 and 9 (Figure 3).

   Describe what happens to the shape of the model when you do this: _____

   _____

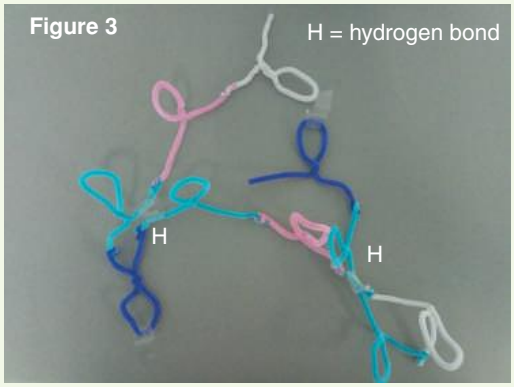
   _____

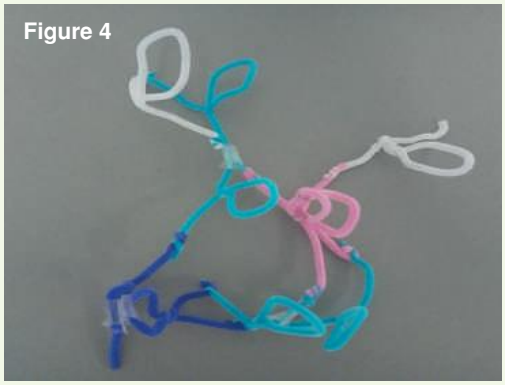
   What level of protein structure does this represent? _____

6. Attach binder clips or paper clips to the loops of the two pink amino acids and then use the clips to join the two pink amino acids together. The clips represent a disulfide bond.

7. Join the sticky tape together on the two purple amino acids (Figure 4). Your protein has now formed its fully functional structure.

   What level of protein structure does this represent? _____





4. (a) Label figure 4 to show the location of all of the hydrogen bonds (H) and the disulfide bond (S).

   (b) Based on the properties of your model and its components, which of these bonds is likely to be the strongest?

   _____

5. Break the hydrogen bonds between amino acids 3 and 5, and also between 7 and 9 in your molecule.

   (a) What happens to the shape of the protein? _____

   _____

   (b) What process does breaking these bonds represent? _____

   _____

   (c) What effect will this process have on the protein's ability to carry out its job? _____

   _____

   _____

   _____

6. How could you adapt your model to demonstrate quaternary structure? _____

   _____

   _____

   _____

   _____

   _____

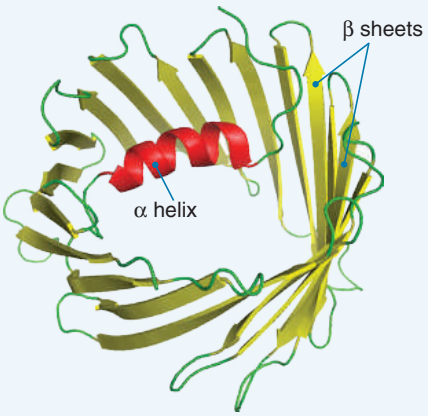
ISBN: 978-1-98-856655-9

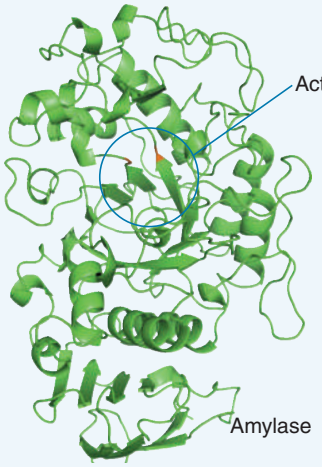
71 Protein Shape is Related to Function

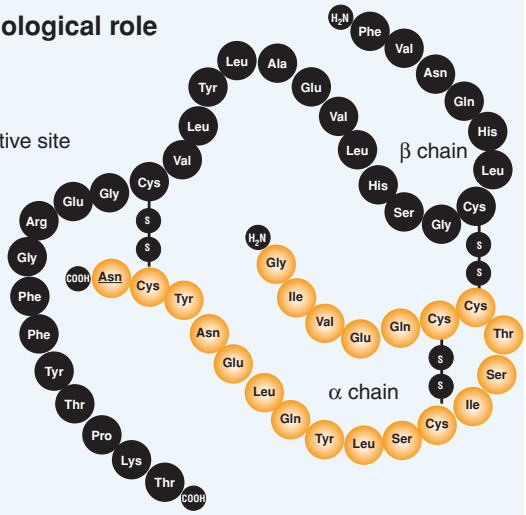
Key Idea: The three dimensional shape of a protein reflects its role. When a protein is denatured, it loses its functionality.

As we have seen, a protein may consist of one polypeptide chain, or several polypeptide chains linked together. Hydrogen bonds between amino acids cause the polypeptide chain to form its **secondary structure**, either an α-helix or a β-pleated sheet. The interaction between R groups causes a polypeptide to fold into its **tertiary structure**, a three dimensional shape held by ionic bonds and disulfide bridges (bonds formed between sulfur-containing amino acids). If bonds are broken through denaturation, the protein loses its tertiary structure, and its functionality.

The shape of a protein reflects its biological role







Channel proteins

Proteins that fold to form channels in the plasma membrane have non-polar R groups facing the membrane and polar R groups facing the inside of the channel. Hydrophilic molecules and ions are then able to pass through these channels into the interior of the cell. Ion channels are found in nearly all cells and many organelles.

Enzymes

Enzymes are globular proteins that catalyse specific reactions. Enzymes that are folded to have polar R groups facing the active site will be specific for polar substances. Non-polar active sites will be specific for non-polar substances. Alteration of the active site by extremes of temperature or pH cause a loss of function.

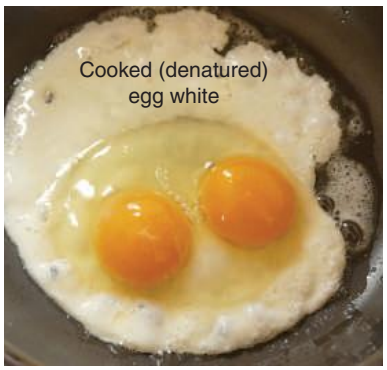
Sub-unit proteins

Many proteins, e.g. insulin and haemoglobin, consist of two or more sub-units in a complex quaternary structure, often in association with a metal ion. Active insulin is formed by two polypeptide chains stabilised by disulfide bridges between neighbouring cysteines. Insulin stimulates glucose uptake by cells.

Protein denaturation

When the chemical bonds holding a protein together become broken the protein can no longer hold its three dimensional shape. This process is called **denaturation**, and the protein usually loses its ability to carry out its biological function.

There are many causes of denaturation, including exposure to heat or pH outside of the protein's optimum range. The main protein in egg white is albumin. It has a clear, thick fluid appearance in a raw egg (right). Heat (cooking) denatures the albumin protein and it becomes insoluble, clumping together to form a thick white substance (far right).



1. Using the example of insulin, explain how interactions between R groups stabilise the protein's functional structure:

2. Why do channel proteins often fold with non-polar R groups to the channel's exterior and polar R groups to its interior?

ISBN: 978-1-98-856655-9

72 Comparing Globular and Fibrous Proteins

Key Idea: The very different structure and properties of globular and fibrous proteins reflect their contrasting roles.

Proteins can be classified according to structure or function. **Globular proteins** such as enzymes are spherical and soluble in water. **Fibrous proteins** have an elongated structure and are not water soluble. They provide stiffness and rigidity to the more fluid components of cells and tissues and have important structural and contractile roles.

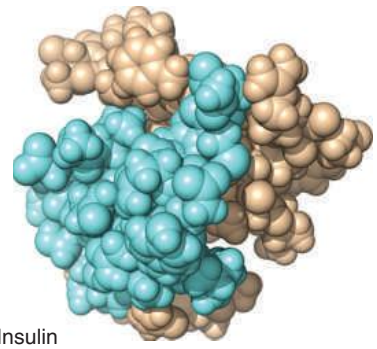
Globular proteins

Properties

- Easily water soluble
- Tertiary structure critical to function
- Polypeptide chains folded into a spherical shape

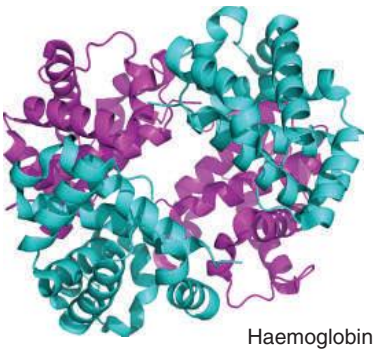
Functions

- Catalytic, *e.g. enzymes*
- Regulatory, *e.g. hormones (insulin)*
- Transport, *e.g. haemoglobin*
- Protective, *e.g. immunoglobulins (antibodies)*



Insulin

RuBisCO



Haemoglobin

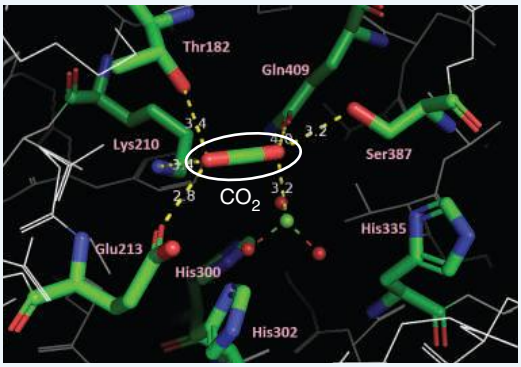
Insulin is a peptide hormone involved in the regulation of blood glucose. Insulin is composed of two peptide chains linked together by two disulfide bonds.

RuBisCo is a large multi-unit enzyme. It catalyses the first step of carbon fixation in photosynthesis. It consists of 8 large and 8 small subunits and is the most abundant protein on Earth.

Haemoglobin is a multi-unit oxygen-transporting protein found in vertebrate red blood cells. One haemoglobin molecule consists of four polypeptide subunits. Each subunit contains an iron-containing haem group, which binds oxygen.

Proteins as catalysts

- Globular proteins act as catalysts for important biological reactions. In this capacity they are enzymes.
- The polypeptide chain folds to produce a site where the reaction occurs. This is called the active site.
- Substrates (chemicals that will react) are drawn into the active site. The reaction occurs, and the products are then released.
- The enzyme is not used up in the reaction but is free to react again. Enzymes are typically named with the suffix *-ase* after the substrate they work on. For example, RuBisCo's full name is *Ribulose-1,5-bisphosphate carboxylase-oxygenase.*



The active site of RuBisCo shown with CO_2 in position

1. What are the functional roles of globular proteins? _____

_____

_____

2. Why is RuBisCo one of the most important proteins on Earth? _____

_____

_____

3. How does the shape of a catalytic protein (enzyme) relate to its functional role? _____

_____

_____

4. What type of protein is haemoglobin? _____

ISBN: 978-1-98-856655-9

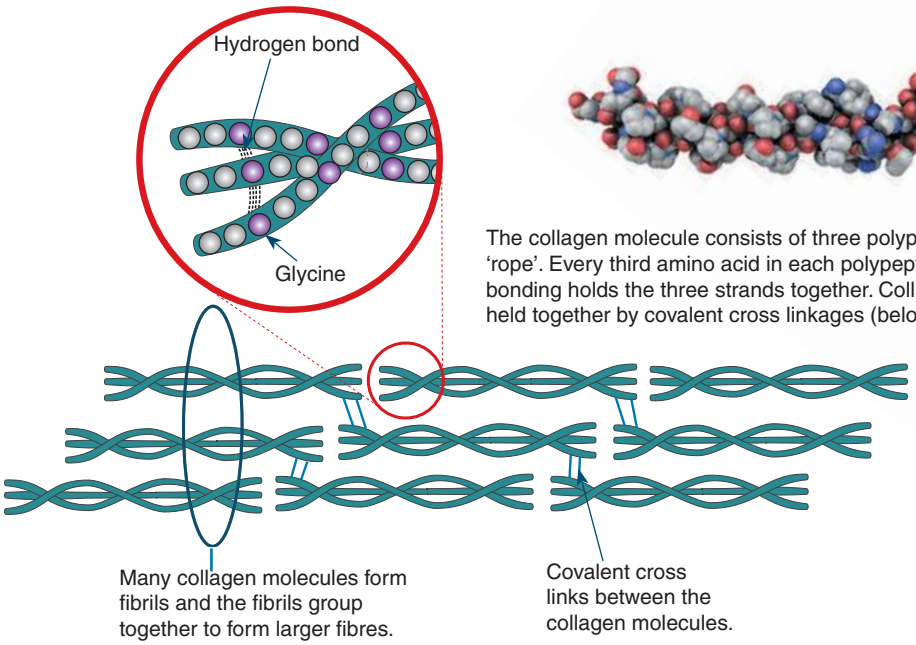
Fibrous proteins

Properties

- Water insoluble
- Very tough physically; may be supple or stretchy
- Parallel polypeptide chains in long fibres or sheets

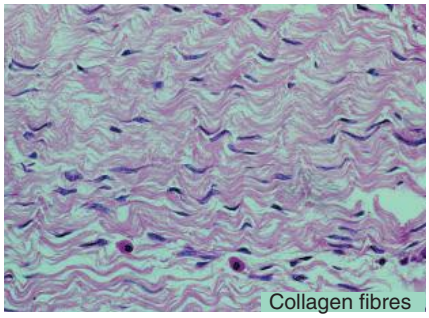
Functions

- Structural role in cells and organisms *e.g. collagen in connective tissues, skin, and blood vessel walls.*
- Contractile *e.g. myosin, actin (muscles)*



Collagen space filling model

The collagen molecule consists of three polypeptides wound together to form a helical 'rope'. Every third amino acid in each polypeptide is a glycine (Gly) where hydrogen bonding holds the three strands together. Collagen molecules self assemble into **fibrils** held together by covalent cross linkages (below). Bundles of fibrils form fibres.

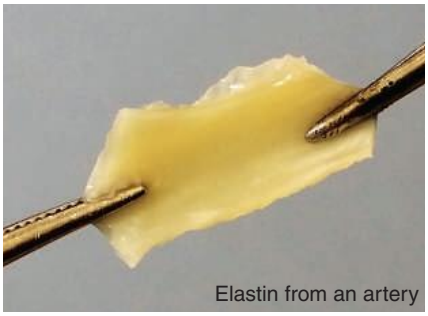


Collagen fibres

Collagen is the main component of connective tissue, and is mostly found in fibrous tissues such as tendons, ligaments, and skin.

Rhinoceros horn is keratin

Keratin is found in hair, nails, horn, hooves, wool, feathers, and the outer layers of the skin. The polypeptide chains of keratin are arranged in parallel sheets held together by hydrogen bonding.



Elastin from an artery

Elastin is found in the extracellular matrix and connective tissue. It allows tissue to stretch, contract, and reform. It is an important component of your skin, helping it reform its shape as you move.

5. (a) What is the role of proteins involved in structural tissues? Give examples to help illustrate your answer:

(b) How does the shape of a fibrous protein relate to its functional role? _____

6. What level of protein structure does collagen have? _____

7. Snake venom is a cocktail of proteins. Some snake venom is so toxic a single bite can kill a human in less than a hour. Some of the proteins act to break down the victim's proteins. What functional group do these kinds of proteins fit into?

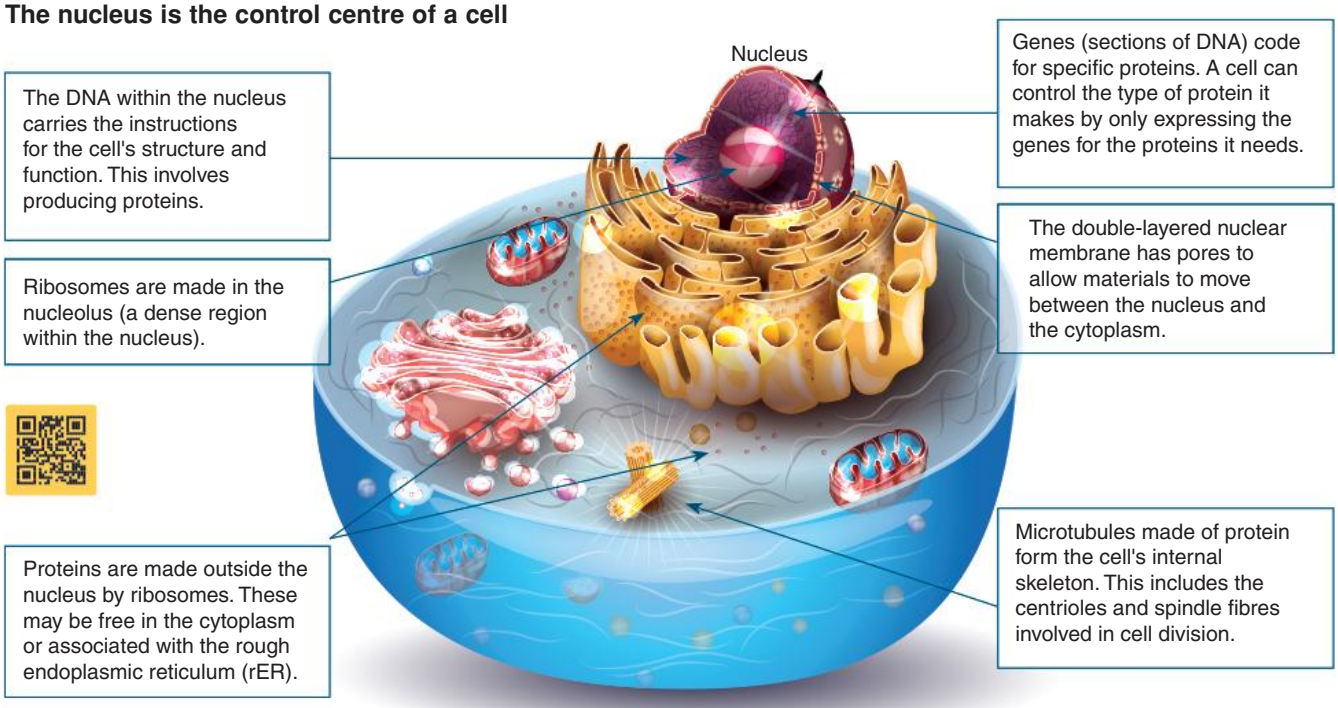
ISBN: 978-1-98-856655-9

73 Protein Functional Diversity

Key Idea: Proteins carry out the essential functions of life and have structural, catalytic, and regulatory roles.

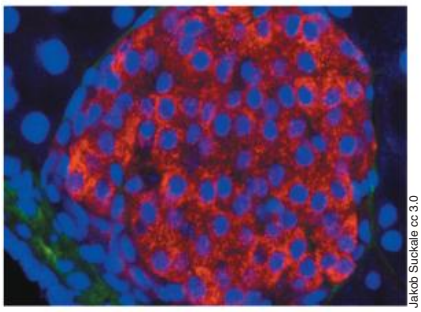
In eukaryotic cells, most of a cell's genetic information (DNA) is found in a large membrane-bound organelle called the nucleus. DNA provides the instructions that code for the formation of proteins. The nucleus directs all cellular activities by controlling the synthesis of proteins, which carry out most of a cell's work. A cell produces many different types of proteins, each with a specific task. Proteins have roles in structure, function, and regulation of the body's cells, tissues, and organs. Without a full complement of functional proteins, a cell can not carry out its specialised role. All of the proteins encoded by an organism's DNA is called its **proteome**. The proteome is larger than the genome because, as you saw earlier, cells are able to produce many different proteins from one set of instructions.

The nucleus is the control centre of a cell



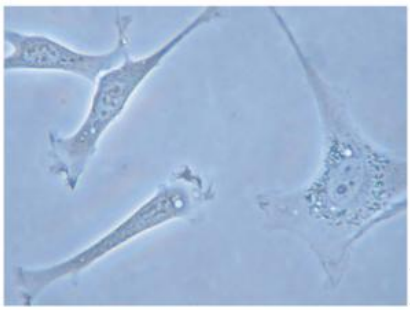
A generalised animal cell

- While a generalised cell produces a range of proteins, some cells in the body are highly specialised to produce large amounts of a specific protein. This specialisation defines their functional role. Three examples are pictured below.



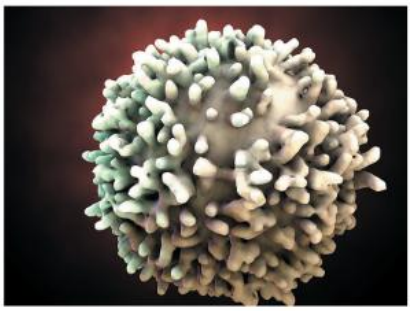
Jakob Suckale cc 3.0

Cells within specialised regions of the pancreas produce and release the protein hormone insulin. Insulin (red in photo) helps to regulate blood glucose.



SubtleGuest cc 2.5

Fibroblasts are specialised cells that continuously produce and secrete the materials that form connective tissue, including the protein collagen.



B lymphocytes (B cells) are white blood cells that are specialised to produce and secrete proteins called antibodies, which protect the body against diseases.

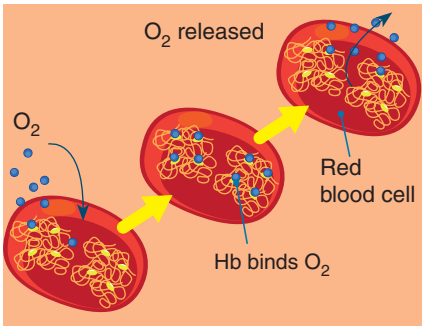
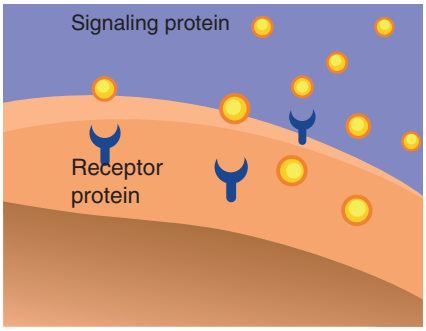
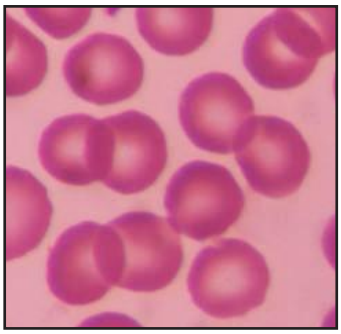
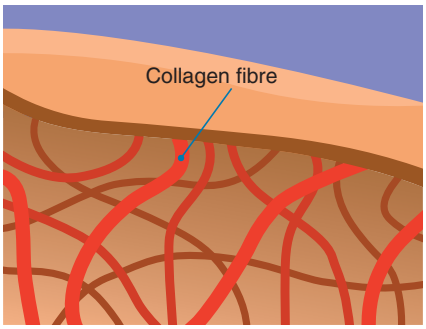
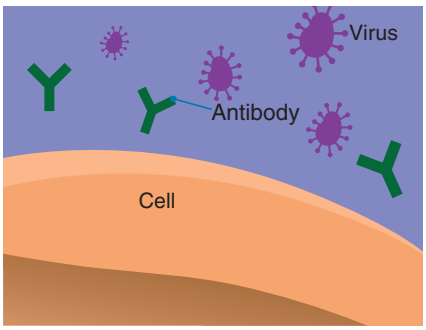
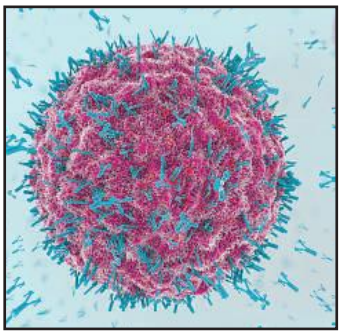
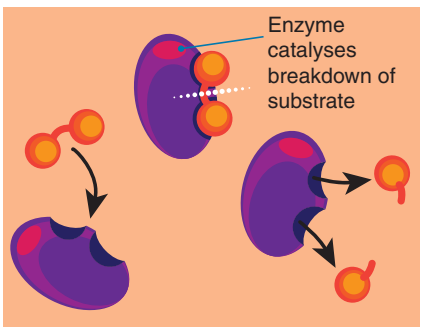
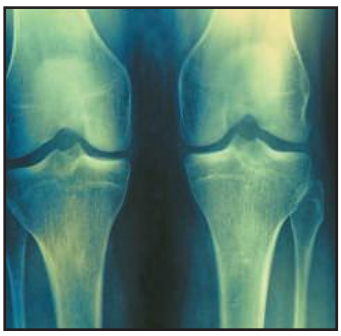
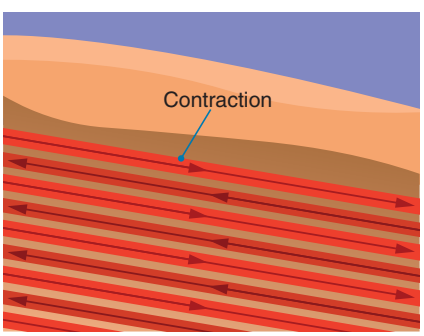
1. Suggest what might happen to a protein's functionality if it was incorrectly encoded by the DNA. Explain your answer:

2. The following pages show six pictograms of proteins in action, six protein functions, six protein examples, and six photographs. These are not in any matched order. Cut out the 24 boxes and paste or tape them into the grid on the next page so that each pictogram is matched with its correct function, example, and illustrative photograph.

ISBN: 978-1-98-856655-9

PICTOGRAM	FUNCTION	EXAMPLE	PHOTO EXAMPLE

ISBN: 978-1-98-856655-9

PICTOGRAM	FUNCTION	EXAMPLE	PHOTO EXAMPLE
	**Internal defence** Antibodies (also called immunoglobulins) are 'Y' shaped proteins that protect the body by identifying and killing disease-causing organisms such as bacteria and viruses.	**Immunoglobulin A** IgA is found in the gut and airways. It destroys disease-causing organisms growing in these areas.	
	**Catalytic** Thousands of different chemical reactions take place in an organism every minute. Each chemical reaction is catalysed by enzymes. The word-ending 'ase' indicates an enzyme.	**Actin & myosin** Two proteins that work together to bring about contraction (movement) in all the muscles of the body, including those that work without your awareness.	
	**Regulation** Regulatory proteins such as hormones act as signal molecules to control biological processes and coordinate responses in cells, tissues, and organs.	**Haemoglobin** A protein found in red blood cells. It binds oxygen and carries it through the blood, delivering it to cells.	
	**Movement** Contractile proteins are involved in movement of muscles and form the internal supporting structures of cells.	**Collagen** Found in the skin and connective tissues, including bones, tendons, and ligaments. It is the most abundant protein in the body.	
	**Transport** Proteins can carry substances across membranes or around the body. In the blood, they transport and store oxygen. In cell membranes they help molecules move into and out of cells.	**Oestrogen** A hormone that is critical for reproduction in females. Oestrogen levels increase during pregnancy to maintain a healthy pregnancy.	
	**Structural** Structural proteins provide physical support or protection. They are strong, fibrous (thread like) and stringy.	**Amylase** An enzyme that breaks down starch into sugars in the first stage of digestion.	

ISBN: 978-1-98-856655-9

THIS PAGE IS DELIBERATELY LEFT BLANK

ISBN: 978-1-98-856655-9

74 How Are Proteins Modified?

Key Idea: The modification of proteins allows the cell to specify their use and final destination.

Proteins may be modified after they have been produced by **post-translational modification**. Two important modifications involve adding carbohydrates or lipids to the protein. **Glycoproteins** are formed by adding carbohydrates to proteins as they pass through the rough endoplasmic reticulum (rER) and Golgi. The carbohydrates may help position and orientate the glycoprotein in the membrane, guide a protein to its final destination, or help in cell-to-cell recognition and cell signalling. Other proteins may have fatty acids added to them in the rER to form **lipoproteins**. These modified proteins transport lipids in the plasma between various organs in the body (e.g. gut, liver, and adipose tissue). Other common post-translational modifications include degradation, cleavage, and phosphorylation (below).

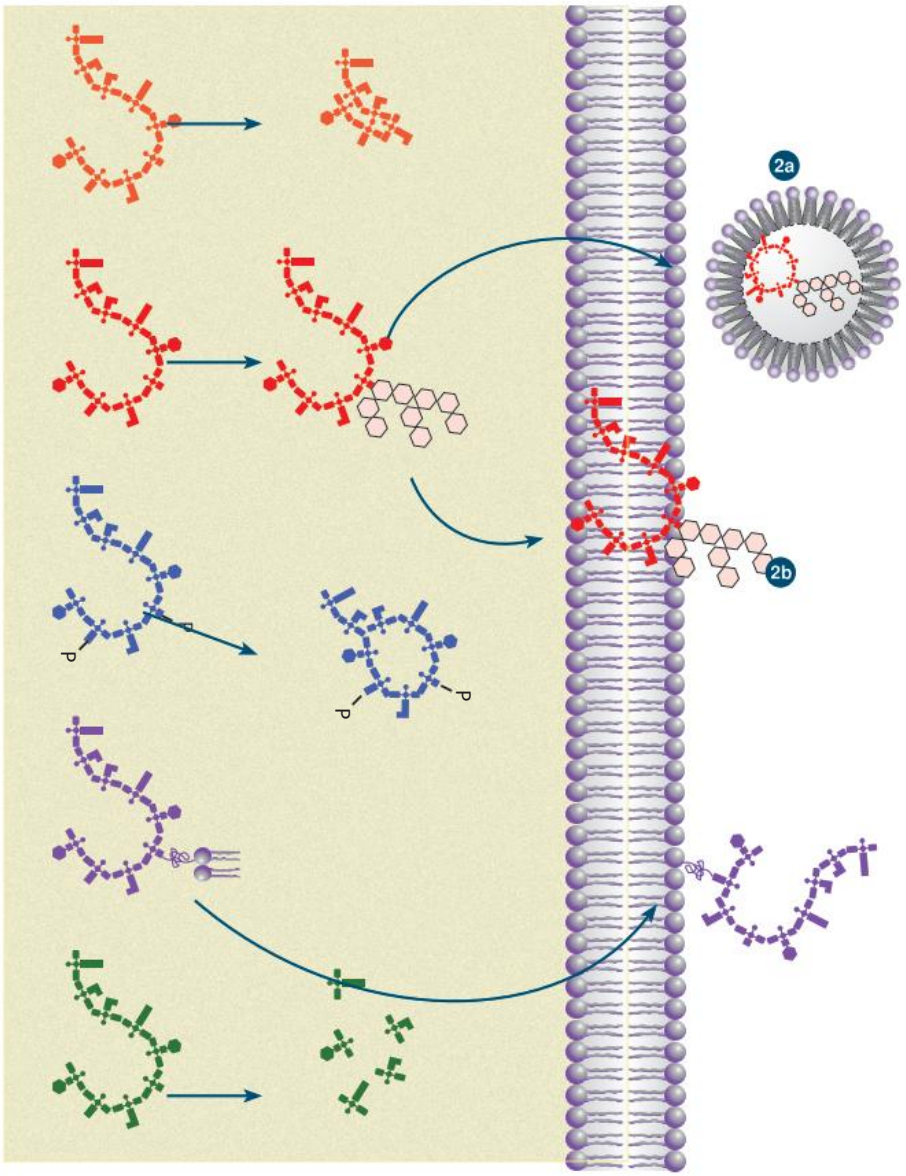
Cleaving: Polypeptide chains may be cleaved to give smaller chains, which then fold or join to give the functional protein. An example is human insulin which is transcribed as one long polypeptide chain which is cleaved to form two shorter chains that form the functional protein.

Glycosylation (adding carbohydrate groups): This is used to add an ID tag to the protein that will allow the cell to recognise its use and where it is to be transported (2a). The resulting glycoprotein may be used in the cell membrane or secreted. The carbohydrate tag may help position the glycoprotein within the membrane (2b).

Phosphorylation (the addition of phosphate groups) takes place in the Golgi. It may contribute to the protein's three dimensional structure or help with cell signalling.

Lipid attachment: Proteins may have lipids attached to them which anchor the protein to the plasma membrane.

Degradation: Some polypeptide chains may be tagged for degradation when they are no longer useful and their amino acids reused in the formation of other proteins.



1. (a) Describe some of the modifications that polypeptide chains undergo before becoming functional proteins:

_____

_____

_____

(b) Why are these changes necessary? _____

_____

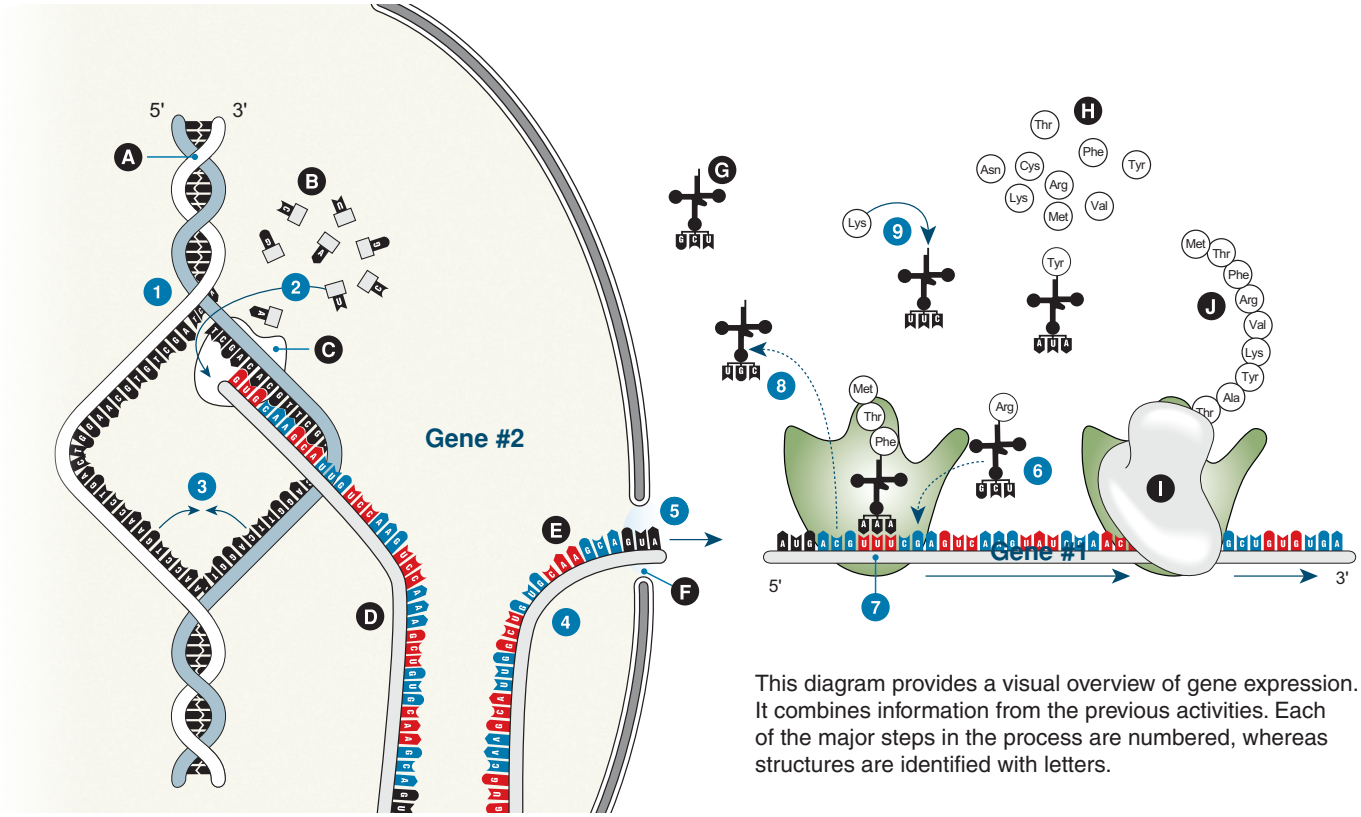
_____

2. Why might the orientation of a protein in the plasma membrane be important? _____

_____

ISBN: 978-1-98-856655-9

75 Chapter Review: Did You Get It?



This diagram provides a visual overview of gene expression. It combines information from the previous activities. Each of the major steps in the process are numbered, whereas structures are identified with letters.

1. Briefly describe each of the numbered processes in the diagram above:

   (a) Process 1: _____

   (b) Process 2: _____

   (c) Process 3: _____

   (d) Process 4: _____

   (e) Process 5: _____

   (f) Process 6: _____

   (g) Process 7: _____

   (h) Process 8: _____

   (i) Process 9: _____

2. Identify each of the structures marked with a letter and write their names below in the spaces provided:

   (a) Structure A: _____ (f) Structure F: _____

   (b) Structure B: _____ (g) Structure G: _____

   (c) Structure C: _____ (h) Structure H: _____

   (d) Structure D: _____ (i) Structure I: _____

   (e) Structure E: _____ (j) Structure J: _____

3. Describe two factors that would determine whether or not a particular protein is produced in the cell:

   (a) _____

   _____

   (b) _____

   _____

ISBN: 978-1-98-856655-9

CHAPTER
4

Genetic Variation

Key terms

allele
allele frequency
autosomal dominant trait
autosomal recessive trait
codominance
continuous variation
dihybrid cross
dominant allele
genetic cross
genotype
heterozygous
homologous pairs
homozygous
incomplete dominance
lethal allele
linked genes
locus
Mendelian inheritance
monohybrid cross
multiple alleles
pedigree
phenotype
probability
Punnett square
qualitative trait
quantitative trait
recessive allele
recombination
sex linkage
trait
X-linkage

Activity number

Inquiry question: *How can the genetic similarities and differences within and between species be compared?*

Genetic variation

Key skills and knowledge

☐	1 Investigate sources of variation. Distinguish between quantitative variation and qualitative variation. Know that quantitative variation results from interactions between multiple genes. Investigate and model meiosis and fertilisation as sources of variation.	76
☐	2 Recall the difference between genes and alleles. Demonstrate understanding of the terms: allele, locus, trait, heterozygous, homozygous, genotype, phenotype.	77
☐	3 Use symbols to represent genotypes for the alleles present at a particular gene locus and distinguish between alleles in genetic crosses.	77 80 81
☐	4 Use Mendel's laws of inheritance to predict outcomes of meiosis in the production of gametes.	79 80 81

Bill Ebbesen CC 3.0

Genetic crosses and inheritance

Key skills and knowledge

☐	5 Understand the rules for calculating probability and apply them to predictions of the genotype and phenotype ratios of genetic crosses.	78
☐	6 Use Punnett squares to model and predict the outcomes of meiosis and fertilisation in relation to autosomal, sex-linkage, codominance, incomplete dominance, lethal, and multiple alleles.	81-84 89
☐	7 Use Punnett squares to predict the frequencies of genotypes and phenotypes, autosomal, sex-linkage, codominance, incomplete dominance, lethal, and multiple alleles.	81-84 89
☐	8 Explain what is meant by genetic linkage (linked genes) and explain its biological consequences. Describe the dihybrid inheritance of linked genes.	85-87
☐	9 Use the chi-squared test to test the outcome of genetic crosses against predicted outcomes. Use the chi-squared test to provide evidence for genotype and linkage.	88
☐	10 Explain what is meant by a sex-linked gene. What are the characteristics of inheritance of X-linked dominant traits? Of X-linked recessive traits?	89
☐	11 Use Punnett squares to determine genotypes and predict the outcomes of genetic crosses involving monohybrid and dihybrid inheritance.	90
☐	12 Analyse pedigree charts to trace the inheritance patterns of particular traits, including autosomal dominant, autosomal recessive, X-linked, and Y-linked traits.	91 92
☐	13 Analyse allele frequencies in populations to determine changes in a gene pool over generations.	93
☐	14 Use mathematical tools, including statistical tests and spreadsheets, to investigate the significance of a quantitative phenotypic change from generation to generation.	94

76 Sources of Variation

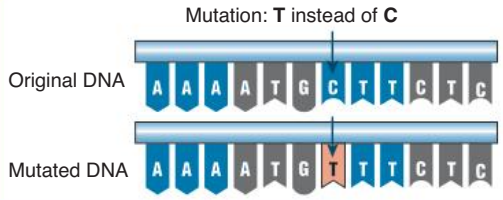
Key Idea: Sexually reproducing organisms show variation in their phenotype (appearance) as a result of both mutation, and the processes involved in meiosis and fertilisation. Genes and environment contribute to the final phenotype.

Variation refers to the diversity of **genotypes** (allele combinations) and **phenotypes** (appearances) in a population. Phenotypic variation occurs in sexually reproducing populations because an organisms's environment affects expression of its genotype. In sexually reproducing organisms, different genotypes account for most of the phenotypic variation we see. Sexual reproduction creates new allele combinations in the offspring due to random fertilisation and meiosis. The new combinations are 'tested' by the environment to see how well they survive and whether they are passed on to the next generation. Variation, as a result of sexual reproduction, provides the raw materials for selecting the best suited phenotypes. This process is called natural selection.

Mutations

Mutations are changes to the DNA

Mutation modifies existing genes to form new alleles. Not all mutations have a phenotypic effect because of the degeneracy of the genetic code. These silent mutations are neither harmful nor beneficial, so may escape selection pressure until conditions change.



Sexual reproduction

Sexual reproduction involves meiosis and fertilisation

Sexual reproduction rearranges and reshuffles the genetic material into new combinations. Fertilisation unites dissimilar gametes to produce more variation.

Don Horne

Genotype

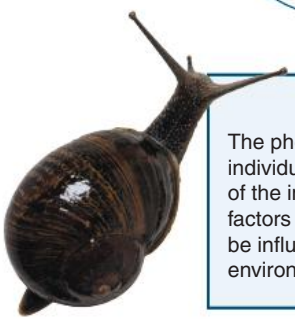
The genetic make up of the individual is its genotype. The genotype determines the genetic potential of an individual.

Gene interactions and epigenetics

Dominant, recessive, codominant, and multiple alleles, epigenetic modifications (such as methylation), and interactions between genes, combine in their effects.

Environmental factors

The environment can influence the expression of the genotype. Both the external environment (e.g. physical and biotic factors) and internal environment (e.g. hormones) can be important.



Phenotype

The phenotype is the physical appearance of an individual. An individual's phenotype is the result of the interaction of genetic and environmental factors during its lifetime. Gene expression can be influenced by both the internal and external environment during and after development.

1. (a) What is the basis of the genetic variation of sexually reproducing organisms? _____

_____

_____

_____

_____

(b) How does the environment contribute to the phenotype we see? _____

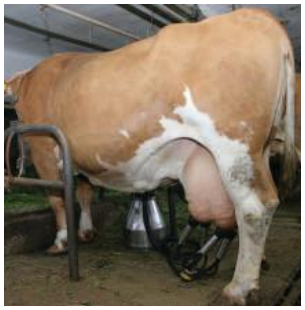
_____

_____

ISBN: 978-1-98-856655-9

What does variation look like?

- Phenotypic variation can be either continuous, with a large number of phenotypic variants approximating a bell shaped (normal) curve, or discontinuous, with only a limited number of phenotypic variants in the population.
- Phenotypic characteristics showing discontinuous variation are determined by only one or two genes, e.g. flower colour.
- Phenotypic characteristics showing continuous variation are determined by a large number of genes and are often heavily influenced by environment, e.g. milk production can be influenced by diet.

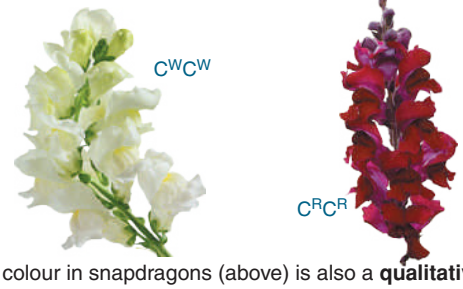



Quantitative traits are characterised by **continuous variation**, with individuals falling somewhere on a normal distribution curve of the phenotypic range. Typical examples include, grain yield in corn (above left), milk production in cattle (centre), and growth in pigs (above, right). Quantitative traits are determined by genes at many loci (polygenic) but most are also influenced by environmental factors.

Albinism (above) is the result of the inheritance of recessive alleles for melanin production. Those with the albino phenotype lack melanin pigment in the eyes, skin, and hair.

Single comb **rrpp** | Walnut comb **R_P_** | Pea comb **rrP_** | Rose comb **R_pp**

Comb shape in poultry is a **qualitative trait** and birds have one of four phenotypes depending on which combination of four alleles they inherit. The dash (missing allele) indicates that the allele may be recessive or dominant.



Flower colour in snapdragons (above) is also a **qualitative trait** determined by two alleles (red and white). The alleles show incomplete dominance and the heterozygote (C^RC^W) exhibits an intermediate phenotype between the two homozygotes (pink).

2. Identify each of the following phenotypic traits as continuous (quantitative) or discontinuous (qualitative):

   (a) Wool production in sheep: _____ (d) Albinism in mammals: _____

   (b) Hand span in humans: _____ (e) Body weight in mice: _____

   (c) Blood groups in humans: _____ (f) Flower colour in snapdragons: _____

3. (a) Distinguish between **continuous** and **discontinuous** variation in phenotype: _____

   _____

   _____

   _____

   (b) Explain the genetic basis of each type: _____

   _____

   _____

   _____

4. Suggest why quantitative traits are more likely to be influenced by the environment than qualitative traits:

   _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

77 Alleles

Key Idea: Eukaryotes generally have paired chromosomes. Each chromosome contains many genes and each gene may have a number of versions called alleles.

Sexually reproducing organisms usually have paired sets of chromosomes, one set from each parent. The equivalent chromosomes that form a pair are termed **homologues**. They carry equivalent sets of genes, but different versions of a gene (**alleles**) can exist in a population.

Homologous chromosomes

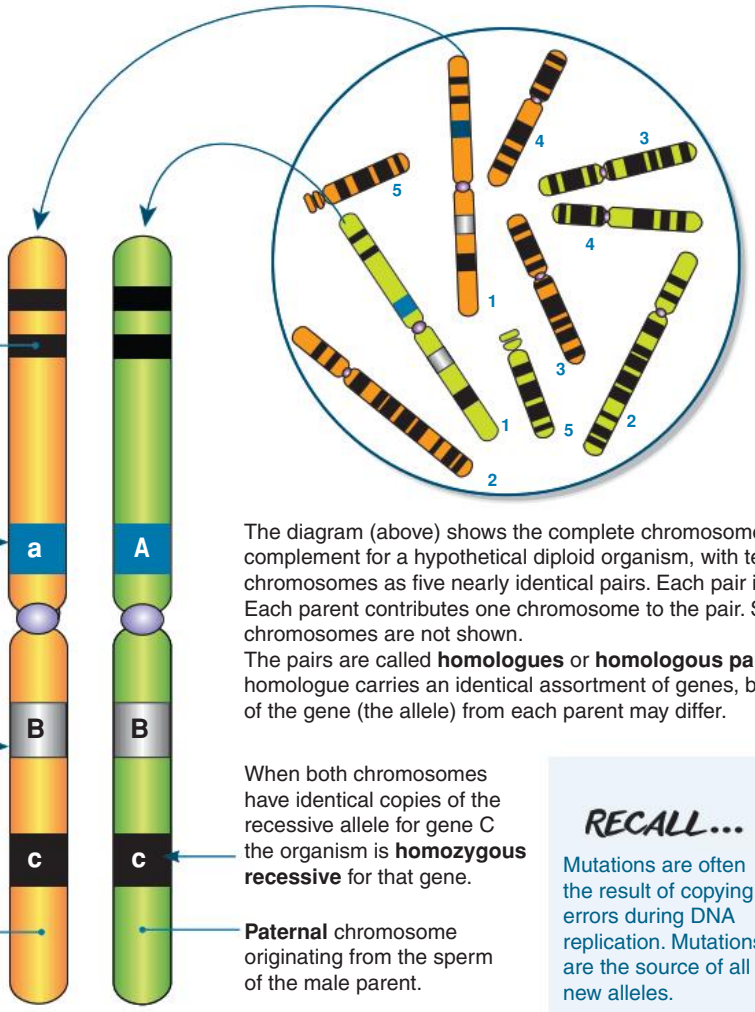
Most cells in sexually reproducing organisms have a homologous pair of chromosomes (one from each parent). Chromosomes are formed from DNA tightly wound around special proteins. This diagram shows the position of three different genes on the same chromosome that control three different traits (A, B and C).

A gene is the unit of heredity. Genes occupying the same **locus** or position on a chromosome code for the same phenotypic character (e.g. eye colour).

Having two different versions (**alleles**) of gene A is called the **heterozygous** condition. Only the dominant allele (A) will be expressed. Alleles differ by only a few bases.

When both chromosomes have identical copies of the dominant allele for gene B the organism is **homozygous dominant** for that gene.

Maternal chromosome originating from the egg of the female parent.



When both chromosomes have identical copies of the recessive allele for gene C the organism is **homozygous recessive** for that gene.

Paternal chromosome originating from the sperm of the male parent.

The diagram (above) shows the complete chromosome complement for a hypothetical diploid organism, with ten chromosomes as five nearly identical pairs. Each pair is numbered. Each parent contributes one chromosome to the pair. Sex chromosomes are not shown.

The pairs are called **homologues** or **homologous pairs**. Each homologue carries an identical assortment of genes, but the version of the gene (the allele) from each parent may differ.

RECALL...

Mutations are often the result of copying errors during DNA replication. Mutations are the source of all new alleles.

1. Define the following terms used to describe the allele combinations in the genotype for a given gene:

   (a) Heterozygous: _____

   (b) Homozygous dominant: _____

   (c) Homozygous recessive: _____

2. For a gene given the symbol 'A', name the alleles present in an organism that is identified as:

   (a) Heterozygous: _____ (b) Homozygous dominant: _____ (c) Homozygous recessive: _____

3. What is a homologous pair of chromosomes? _____

4. Discuss the significance of genes existing as alleles: _____

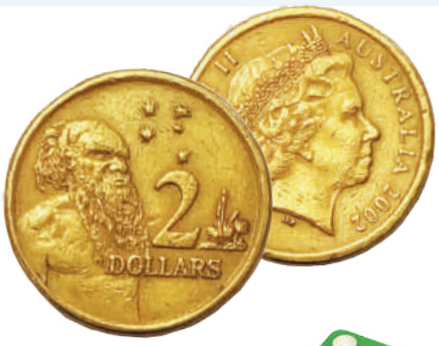
ISBN: 978-1-98-856655-9

78 Probability in Genetics

Key Idea: Probability can be used to determine how likely it is that an event will occur, or to quantify the statistical difference between means.
We cannot always predict events with absolute certainty, but we can use **probability** to calculate the likelihood of an event occurring. The probability of an event ranges from 0 to 1. The sum of all probabilities equals 1. In biology, probability is used to determine the statistical significance of an outcome or the probability of an event occurring, e.g. getting an offspring with a certain genotype and phenotype in a genetic cross.

Probability of an event happening =

$$\frac{\text{Number of ways it can happen}}{\text{Total number of outcomes}}$$



- Tossing a coin and predicting whether it will land heads (H) up or tails (T) up is a good example to illustrate probability.
- There are two possible outcomes. The coin will either land heads up or tails up, and only one outcome can occur at a time. The probability of a coin landing heads up is 1/2. The probability of a coin landing tails up is also 1/2.
- Remember that probability is just an indication of how likely something will happen. Even though we predict that heads and tails will come up 50 times each if we toss a coin 100 times, it might not be exactly that.

1. Calculate the probability of throwing a 6 when you roll a die (plural, dice):

The rules for calculating probability

- Probability rules are used when we want to predict the likelihood of two events occurring together or when we want to determine the chances of one outcome over another.
- The rules are useful when we want to determine the probably of certain outcomes in genetic crosses, especially when large numbers of alleles are involved.
- The probability rule used depends on the situation.

PRODUCT RULE for independent events

For independent events, A & B, the probability (P) of them both occurring (A&B) = P(A) X P(B)

Example: If you roll two dice at the same time, what is the probability of rolling two sixes?
Solution: The probability of getting six on two dice at once is 1/6 x 1/6 = 1/36.

SUM RULE for mutually exclusive events

For mutually exclusive events, A & B, the probability (P) that one will occur (A or B) = P(A) + P(B)

Example: A single die is rolled. What are the chances of rolling a 2 **or** a 6?
Solution: P(A or B) = P(A) + P(B). 1/6 + 1/6 = 2/6 (1/3). There is a 1/3 chance that a 2 or 6 will be rolled.

2. In a cross Aa x Aa, use the sum rule to determine the probability of the offspring having a dominant phenotype:

3. Use the product rule to determine the probability of a first and second child born to the same parents both being boys:

4. In a cross of rabbits, both heterozygous for genes for coat colour and length (BbLl x BbLl), determine the probability of the offspring being BbLl.
HINT: Calculate probabilities for Bb and Ll separately and then use the product rule. Test your calculation using the Punnett square (right).

5. In a cross of two individuals with various alleles of four unlinked genes: AaBbCCdd x AabbCcDd, explain how you would calculate the probability of obtaining offspring with the dominant phenotype for all four traits:

ISBN: 978-1-98-856655-9

79 Mendel's Laws of Inheritance

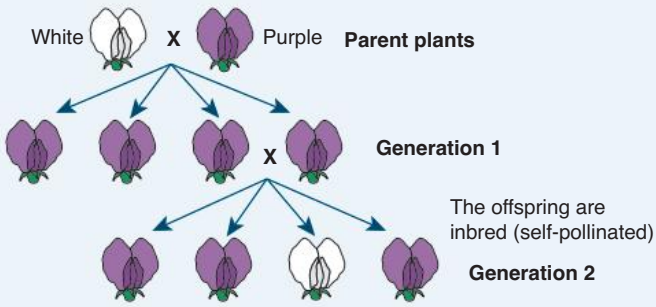
Key Idea: Genetic information is inherited from parents in discrete units called genes.

Mendel's laws of inheritance were based on his observations and govern how genes are passed to the offspring.

Particulate inheritance

Characteristics of both parents are passed on to the next generation as discrete entities (genes).

This model explained many observations that could not be explained by the idea of blending inheritance, which was universally accepted prior to the particulate inheritance theory. The trait for flower colour (right) appears to take on the appearance of only one parent plant in the first generation, but reappears in later generations.

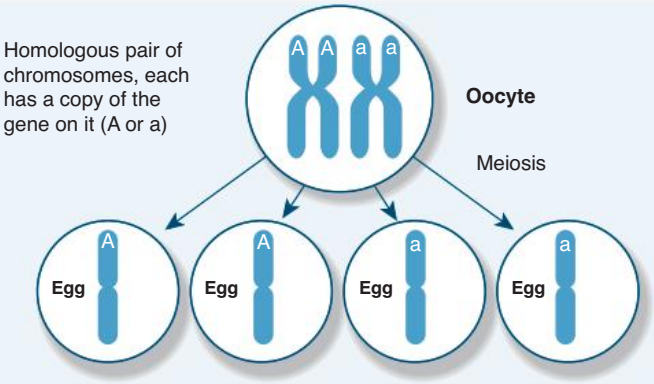


Law of segregation

When gametes are being produced during meiosis only one of the two copies of the gene is passed to each gamete.

These gametes are eggs (ova) and sperm cells. The allele in the gamete will be passed on to the offspring.

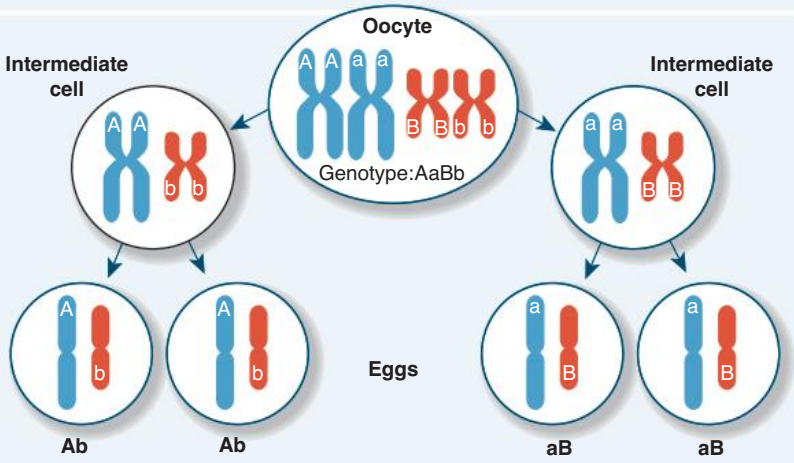
NOTE: This diagram has been simplified, omitting the stage where the second chromatid is produced for each chromosome.



Law of independent assortment

Allele pairs separate independently during gamete formation, and traits are passed on to offspring independently of one another (this is only true for unlinked genes).

This diagram shows two genes, A and B, that code for different traits. Each of these genes is represented twice, one copy (allele) on each of two homologous chromosomes. The genes A and B are located on different chromosomes and, because of this, they will be inherited independently of each other i.e. the gametes may contain any combination of the parental alleles.



1. State the **property of genetic inheritance** that allows parent pea plants of different flower colour to give rise to flowers of a single colour in the first generation, with both parental flower colours reappearing in the following generation:

_____

_____

2. The oocyte is the egg producing cell in the ovary of an animal. In the diagram illustrating the **law of segregation** above:

   (a) State the genotype for the oocyte (adult organism): _____

   (b) State the genotype of each of the **four** gametes: _____

   (c) State how many different kinds of gamete can be produced by this oocyte: _____

3. The diagram illustrating the **law of independent assortment** (above) shows only one possible result of the random sorting of the chromosomes to produce Ab and aB in the gametes.
   (a) List another possible combination of genes (on the chromosomes) ending up in gametes from the same oocyte:

   _____

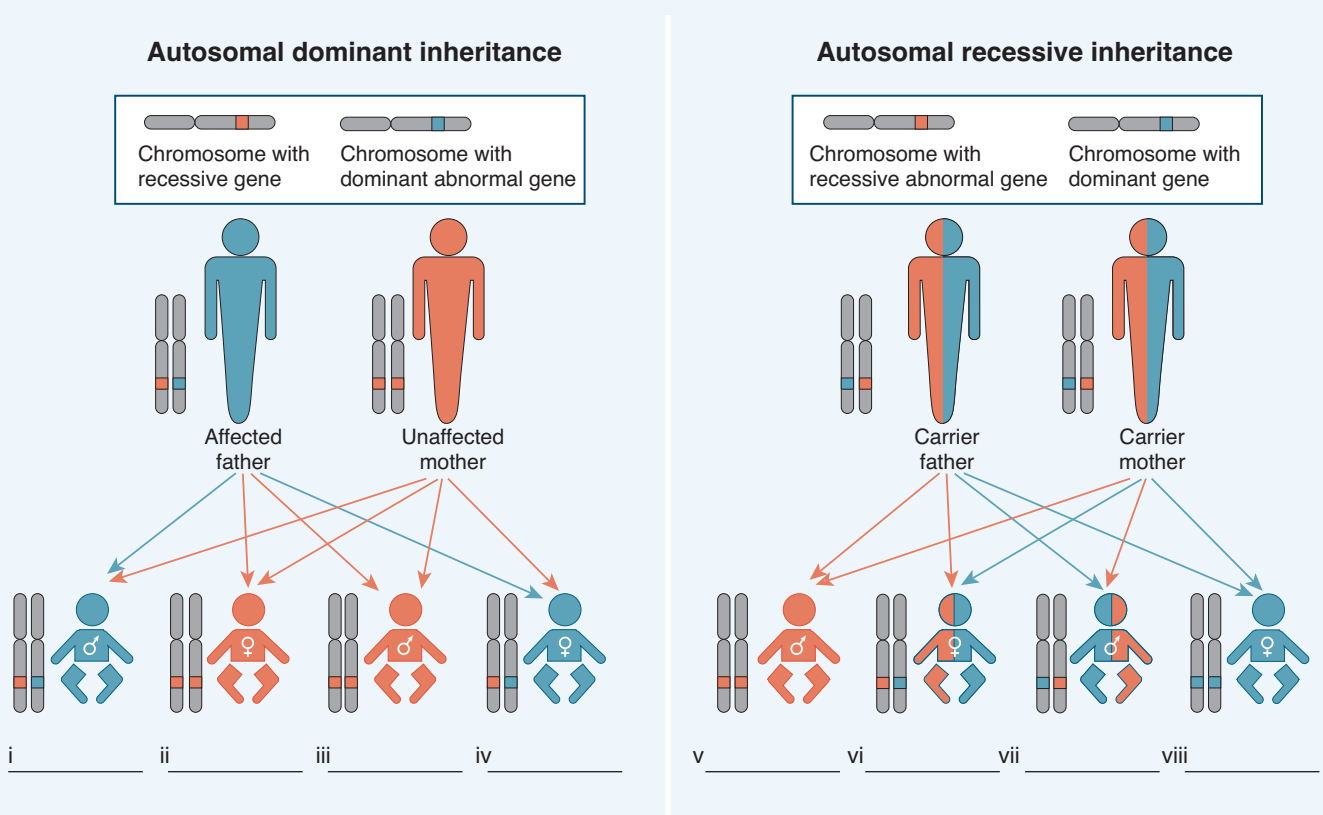
   (b) How many different gene combinations are possible for the oocyte? _____

ISBN: 978-1-98-856655-9

80 Autosomal Dominant and Recessive Inheritance

Key Idea: Autosomal dominant traits are always expressed regardless of whether there are one or two copies of the allele.

An autosome is any chromosome that is not a sex chromosome so an autosomal gene is found on an autosome. A dominant allele will always be expressed in the phenotype regardless of whether the individual has one copy (heterozygous) or two copies (homozygous) of the dominant allele. An autosomal dominant condition is easily recognised because people with only one copy of the allele will be affected and show symptoms. In an autosomal recessive condition, two copies of the gene must be present for the disease to occur. Individuals with only one copy of a gene are carriers, they don't have the disease but can pass the gene on to their offspring.



1. Explain the inheritance pattern of an autosomal dominant allele: _____

2. Fill in the spaces (i-viii) in the diagram above to identify the children as **affected**, **carrier**, or **unaffected**. A carrier carries the mutated allele but does not show any symptoms of the disease.

3. The diagram (above left) shows inheritance when the father is heterozygous for the affected allele. Describe what the phenotype of the offspring would be if the father had been homozygous for the affected allele:

4. Contrast the pattern of inheritance for an autosomal dominant and an autosomal recessive condition:

5. Some diseases caused by autosomal dominant alleles, such as Huntington's disease, do not become apparent until well into adulthood. Suggest how this might explain why such diseases persist in the population:

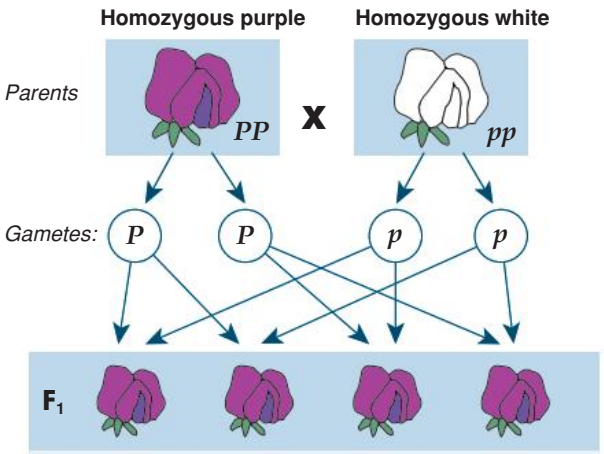
ISBN: 978-1-98-856655-9

81 Basic Genetic Crosses

Key Idea: The outcome of a cross depends on the parental genotypes and can be predicted using Punnett squares.

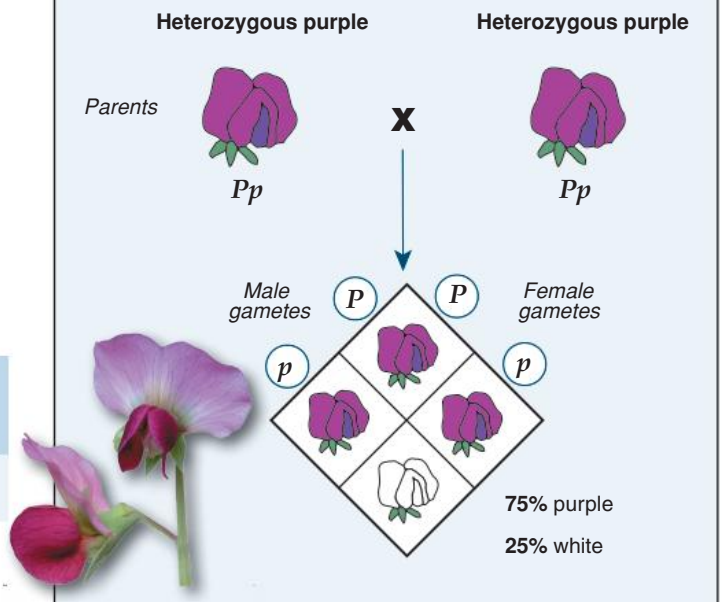
Examine the diagrams below on monohybrid (one gene) and dihybrid (two gene) inheritance. The F_1 generation describes the offspring of a cross between **true-breeding** (homozygous) parents. A **back cross** is a cross between an offspring and one of its parents. If the back cross is to a homozygous recessive, it can be used as a test cross.

Monohybrid cross F_1



A true-breeding organism is homozygous for the gene involved. The F_1 offspring of a cross between two **true breeding** parent plants are all purple (*Pp*).

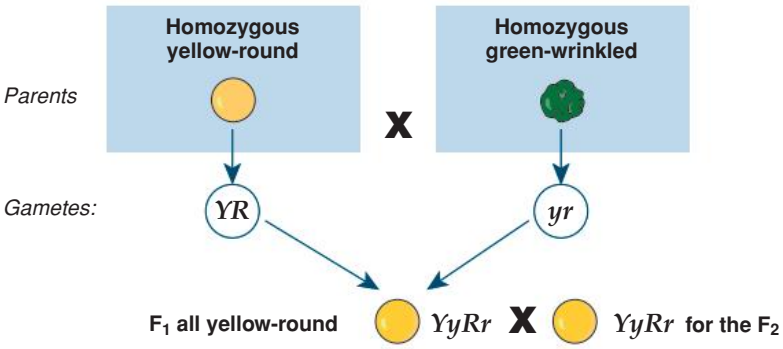
Monohybrid cross F_2



A cross between the F_1 offspring (*Pp x Pp*) would yield a 3:1 ratio in the F_2 of purple (*PP, Pp, Pp*) to white (*pp*).

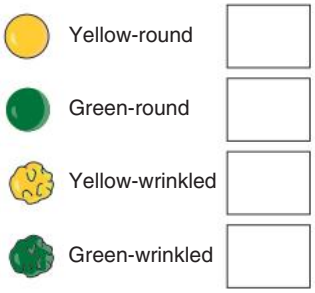
Dihybrid cross

A dihybrid cross studies the inheritance patterns of two genes. In pea seeds, yellow colour (*Y*) is dominant to green (*y*) and round shape (*R*) is dominant to wrinkled (r). Each **true breeding** parental plant has matching alleles for each of these characters (*YYRR* or *yyrr*). F_1 offspring will all have the same genotype and phenotype (yellow-round: *YyRr*).



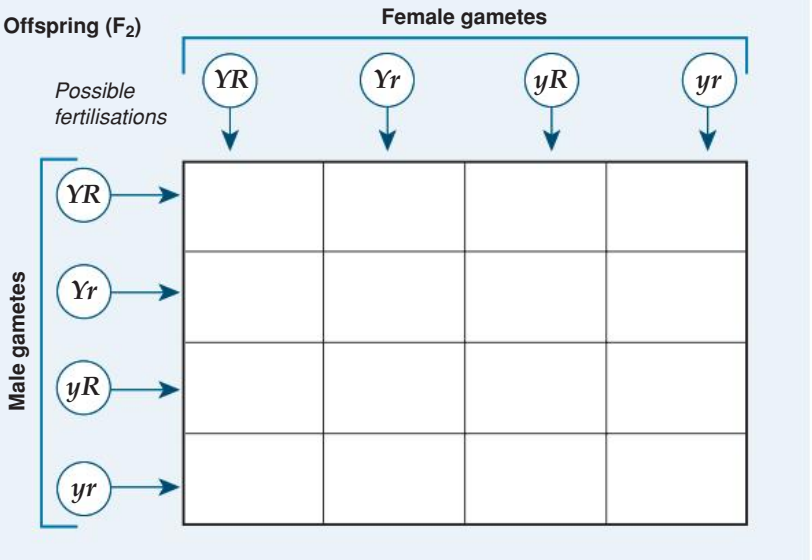
1. Fill in the Punnett square (below right) to show the genotypes of the F_2 generation.

2. In the boxes below, use fractions to indicate the numbers of each phenotype produced from this cross.



3. Express these numbers as a ratio:

_____

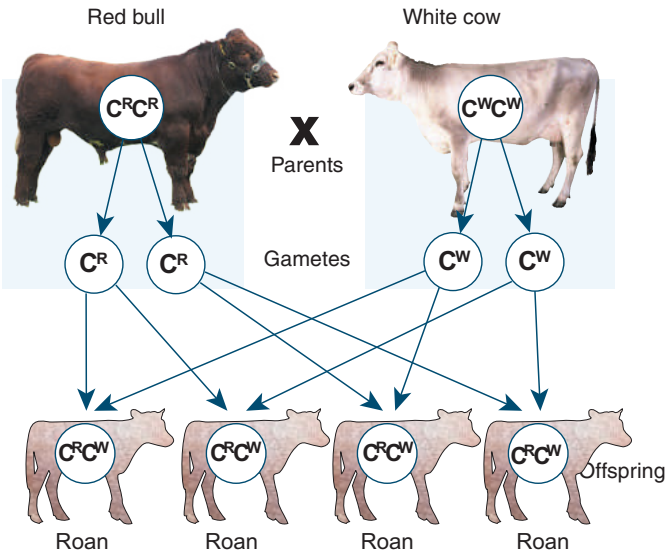


ISBN: 978-1-98-856655-9

82 Codominance

Key Idea: In the inheritance of codominant alleles, neither allele is recessive. Both alleles are equally and independently expressed in the heterozygote.

Codominance is an inheritance pattern in which both alleles in a heterozygote contribute to the phenotype, and both alleles are independently and equally expressed. Examples include the human blood group AB, and certain coat colours in horses and cattle. Reddish coat colour is equally dominant with white. Animals that have both alleles have coats that are roan (both red and white hairs are present).




A roan shorthorn heifer

In the shorthorn cattle breed, coat colour is inherited. White shorthorn parents always produce calves with white coats. Red parents always produce red calves. However, when a red parent mates with a white one, the calves have a coat colour that is different from either parent; a mixture of red and white hairs, called roan. Use the example (left) to help you to solve the problems below.

1. Explain how codominance of alleles can result in offspring with a phenotype that is different from either parent:

_____

2. A white bull is mated with a roan cow (right):

   (a) Fill in the spaces to show the genotypes and phenotypes for parents and calves:

   (b) What is the phenotype ratio for this cross?

   _____

   (c) How could a cattle farmer control the breeding so that the herd ultimately consisted of only red cattle?

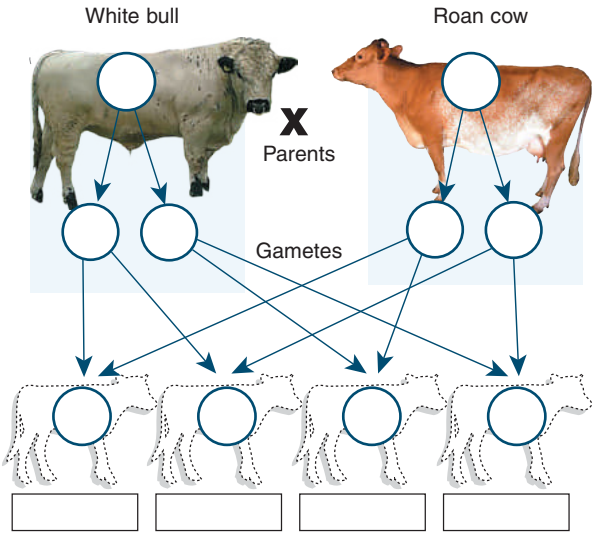
   _____

   _____

   _____

   _____

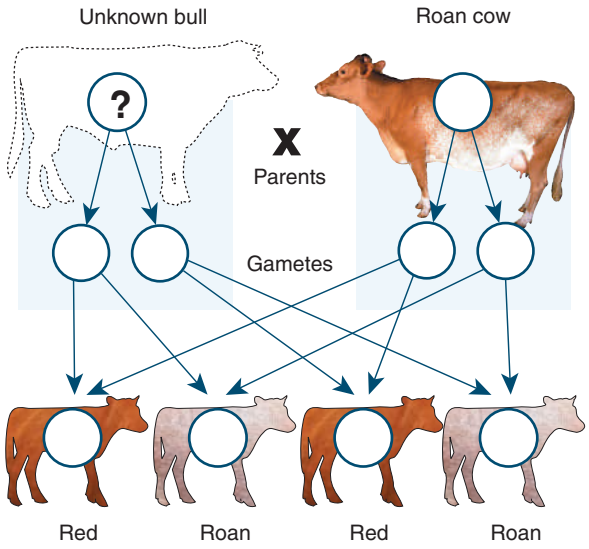
   _____



3. A farmer has only roan cattle on his farm. He suspects that one of the neighbours' bulls may have jumped the fence to mate with his cows earlier in the year because half the calves born were red and half were roan. One neighbour has a red bull, the other has a roan.

   (a) Fill in the spaces (right) to show the genotype and phenotype for parents and calves.

   (b) Which bull serviced the cows? Red or roan (*delete one*)



4. Describe the classical phenotypic ratio for a codominant gene resulting from the cross of two heterozygous parents, e.g. a cross between two roan cattle:

_____

_____

ISBN: 978-1-98-856655-9

- The human ABO blood group system also shows codominance. The four common blood groups of the human 'ABO blood group system' are determined by three alleles: A, B, and O. The ABO antigens consist of sugars attached to the surface of red blood cells. The alleles code for enzymes (proteins) that join these sugars together.
- The allele O is recessive. It produces a non-functioning enzyme that cannot make any changes to the basic sugar molecule.
- The other two alleles (A, B) are codominant and are expressed equally. They each produce a different functional enzyme that adds a different, specific sugar to the basic sugar molecule.
- The blood group A and B antigens are able to react with antibodies present in the blood of other people so blood must always be matched for transfusion.

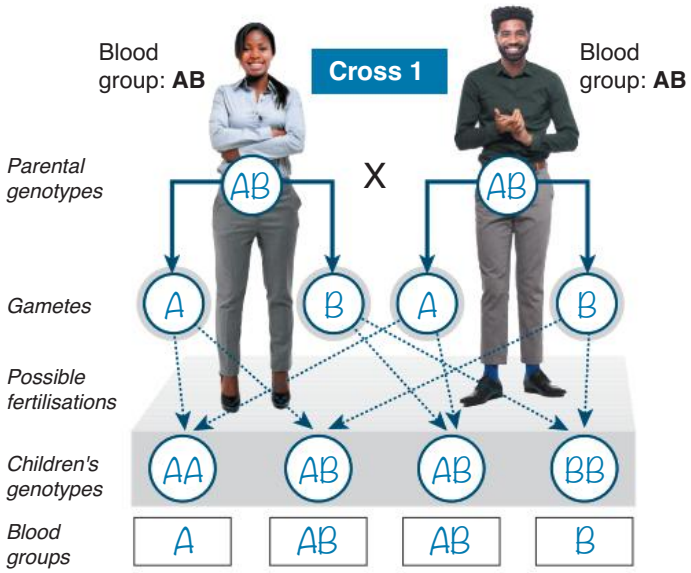
Recessive allele: ***O*** produces a non-functioning protein
Dominant allele: ***A*** produces an enzyme which forms **A antigen**
Dominant allele: ***B*** produces an enzyme which forms **B antigen**

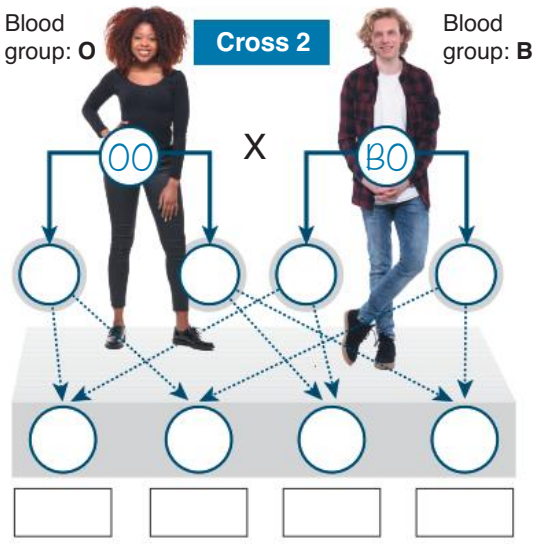
If a person has the ***AO*** allele combination then their blood group will be group **A**. The presence of the recessive allele has no effect on the blood group in the presence of a dominant allele. Another possible allele combination that can create the same blood group is ***AA***.

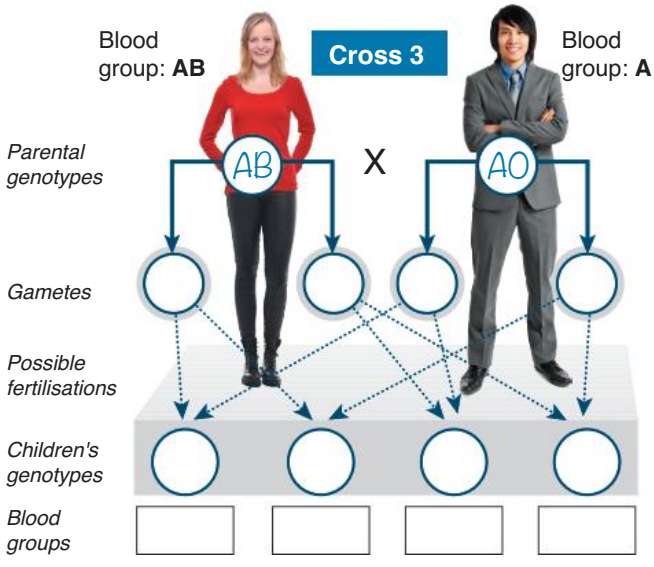
Blood group (phenotype)	Possible genotypes	Frequency in Australia
O	OO	49%
A	AA AO	38%
B		10%
AB		3%

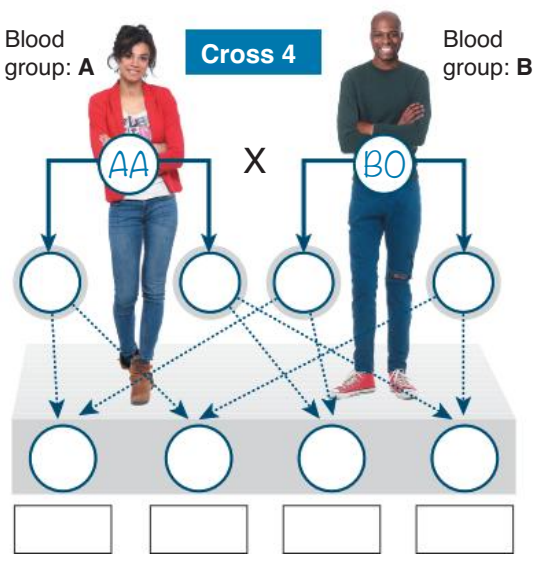
5. Use the information above to complete the table for the possible genotypes for blood group B and group AB.

6. Below are four crosses possible between couples of various blood group types. The first example has been completed for you. Complete the genotype and phenotype for the other three crosses below:





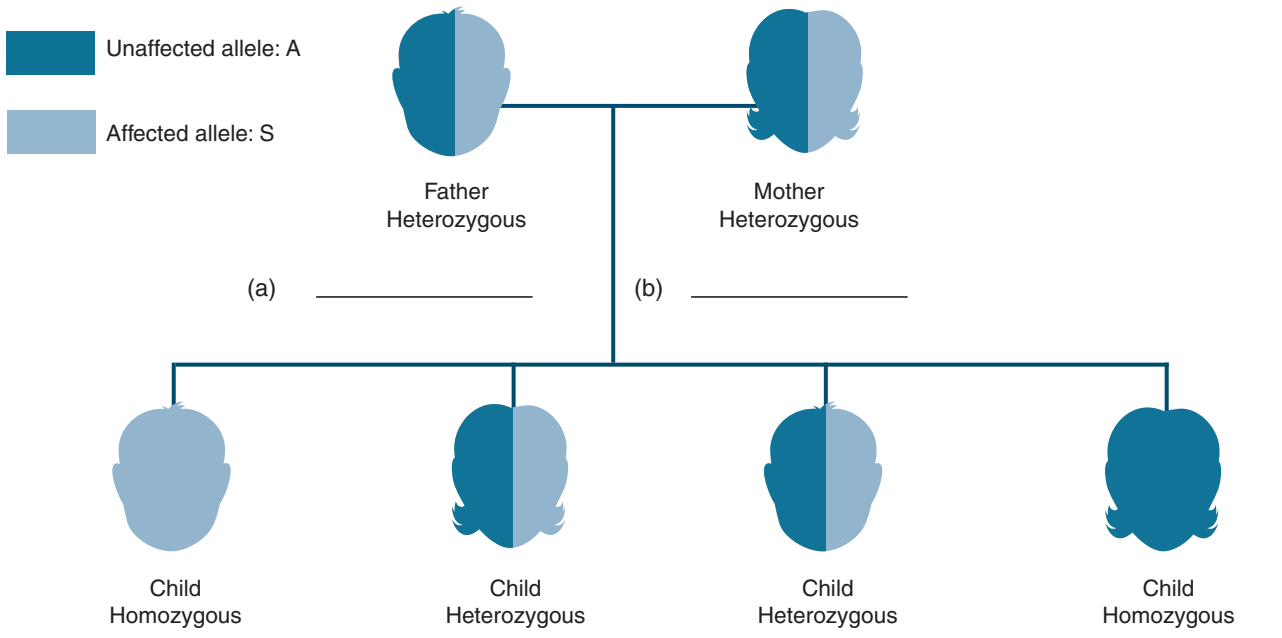




ISBN: 978-1-98-856655-9

7. Sickle cell disease is a condition inherited in an autosomal recessive pattern (although the alleles are codominant). Two copies of the recessive allele must be inherited for an individual to have sickle cell anemia. An individual with only one copy is a carrier for sickle cell trait (they have both normal and sickled RBCs). The inheritance pattern for two parents heterozygous for the sickle cell mutation is shown below.

   Write the phenotype and genotype (AA, AS, or SS) for each individual on the diagram below:



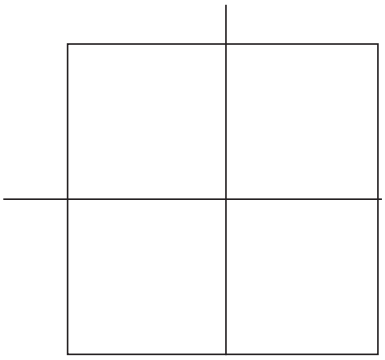
8. (a) Complete the Punnett square (right) for individuals with the following genotypes (AS and AA):

   (b) State all the possible phenotypes from this cross: _____

   _____

   (c) What is the probability of producing offspring that are carriers?

   _____



9. People with sickle cell disease used to die early in life from medical complications. Many now live into their 40s and 50s because of medical advances.

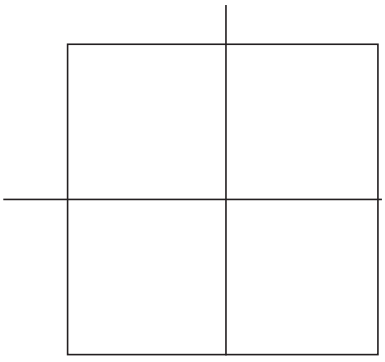
   (a) Complete the Punnett square (right) for individuals with the following genotypes (AS and SS):

   (b) What is the probability that these individuals would produce a child with sickle cell disease?

   _____

   (c) What is the probability that these individuals would produce a child that was AS (a carrier)?

   _____



10. Parents produced children who were all heterozygotes for the sickle cell allele.

    (a) What is the likely genotype of each parent? _____

    (b) Can you be 100% sure of this? If not, why not? _____

    _____

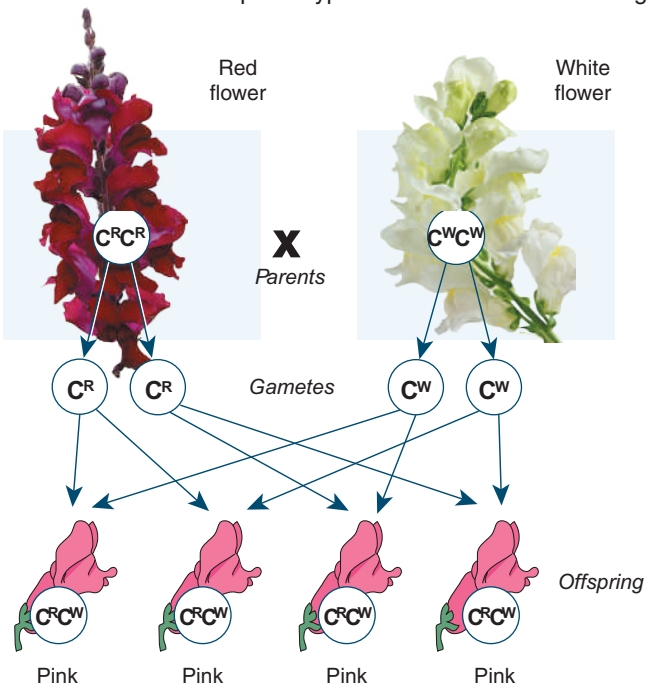
    _____

ISBN: 978-1-98-856655-9

83 Incomplete Dominance

Key Idea: Incomplete dominance describes the situation where the action of one allele does not completely mask the action of the other and neither allele shows dominance in determining the trait.

In incomplete dominance the heterozygous offspring are intermediate in phenotype between the contrasting homozygous parental phenotypes. In crosses involving incomplete dominance, the phenotype and genotype ratios are identical. The phenotype of heterozygous offspring results from the partial influence of both alleles. Examples of incomplete dominance includes flower colour in snapdragons (*Antirrhinum*) and four o'clocks (*Mirabilis*) (below).



Pure breeding snapdragons produce red or white flowers (left). When red and white-flowered parent plants are crossed a pink-flowered offspring is produced. If the offspring (F_1 generation) are then crossed together, all three phenotypes (red, pink, and white) are produced in the F_2 generation.

Four o'clocks (above) are also known to have flower colours controlled by alleles that show incomplete dominance. Pure breeding four o'clocks produce crimson, yellow, or white flowers. Crimson flowers (above) crossed with yellow flowers produced reddish-orange flowers, while crimson flowers crossed with white flowers produce magenta (reddish-pink) flowers.

1. Explain how incomplete dominance of alleles differs from complete dominance: _____

_____

_____

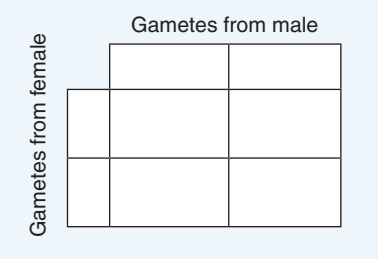
_____

2. A plant breeder wanted to produce snapdragons for sale that were only pink or white (i.e. no red). Determine the phenotypes of the two parents necessary to produce these desired offspring. Use the Punnett square (right) to help you:

_____

_____

_____



3. Another plant breeder crossed two four o'clocks, known to have flower colour controlled by alleles that show incomplete dominance. Pollen from a magenta flowered plant was placed on the stigma of a crimson flowered plant.

   (a) Fill in the spaces on the diagram on the right to show the genotype and phenotype for parents and offspring.

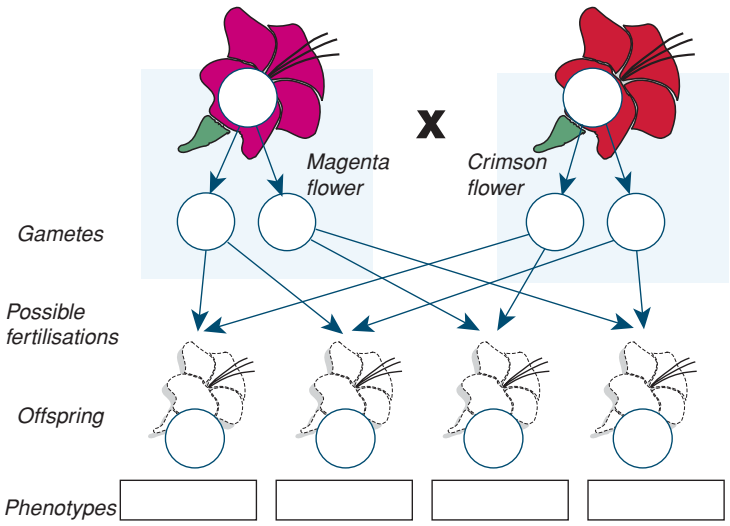
   (b) State the phenotype ratio:

   _____

   _____

   _____

   _____



ISBN: 978-1-98-856655-9

84 Lethal Alleles

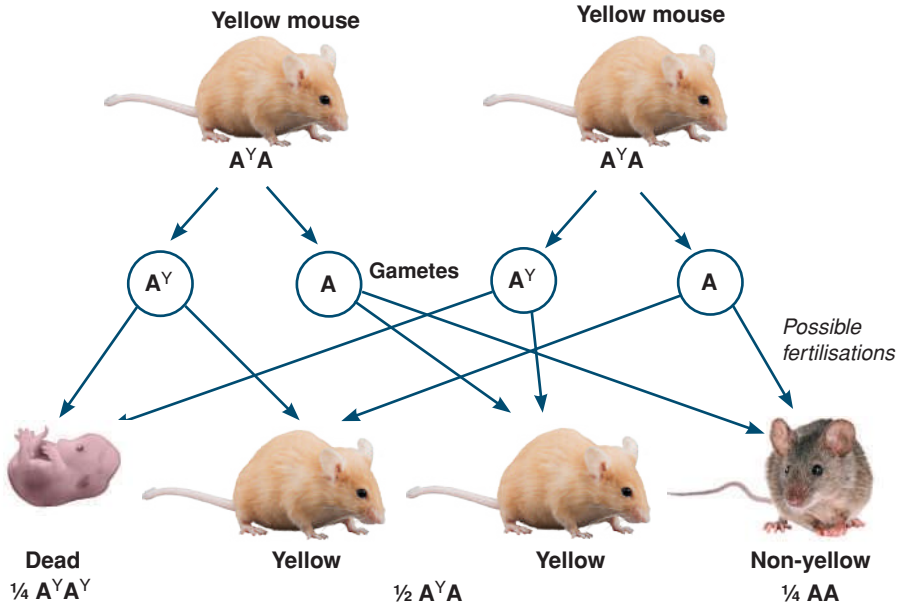
Key Idea: Phenotypic outcomes involving lethal alleles do not follow expected Mendelian ratios.

Lethal alleles are usually a result of mutations in essential genes. They may result in death of an organism because an essential protein is not produced. Some lethal alleles are lethal in both homozygous dominant and heterozygous conditions. Others are lethal only in the homozygous condition (either dominant or recessive). Furthermore, lethal alleles may take effect at different stages in development, e.g. symptoms of Huntington's disease usually appear after 30 years of age.

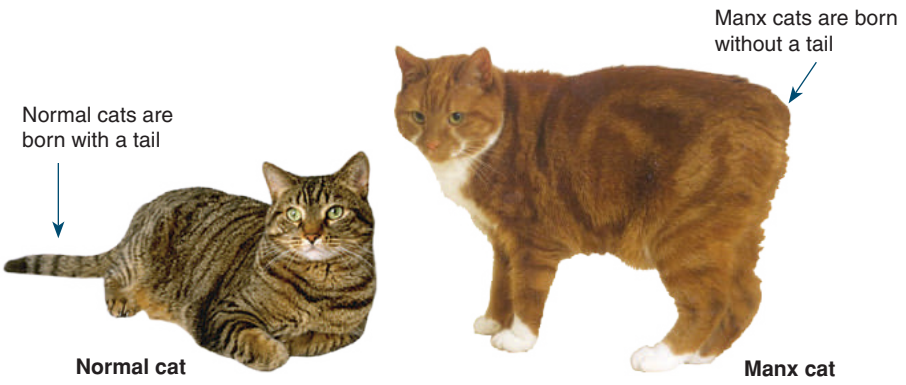
When Lucien Cuenot investigated inheritance of coat colour in yellow mice in 1905, he reported a peculiar pattern.

When he mated two yellow mice, about 2/3 of their offspring were yellow, and 1/3 were non-yellow (a 2:1 ratio). This was a departure from the expected Mendelian ratio of 3:1.

A test cross of the yellow offspring showed that all the yellow mice were heterozygous. No homozygous dominant yellow mice were produced because they had two copies of a lethal allele (Y). The Y allele is a mutation of the wild type agouti gene (A).



Cats possess a gene for producing a tail. The tailless **Manx** phenotype in cats is produced by an allele that is lethal in the homozygous state, as the Manx allele $\mathbf{M^L}$ severely interferes with normal spinal development. In $\mathbf{M^LM^L}$ homozygotes, the double dose of the gene produces an extremely abnormal embryo, which does not survive. In heterozygotes ($\mathbf{M^LM}$), it results in the absence of a tail.



1. In Manx cats, the allele for taillessness ($\mathbf{M^L}$) is incompletely dominant over the recessive allele for normal tail (M). Tailless Manx cats are heterozygous ($\mathbf{M^LM}$) and carry a recessive allele for normal tail. Normal tailed cats are MM. A cross between two Manx (tailless) cats, produces two Manx to every one normal tailed cat (not a regular 3 to 1 ratio).

   (a) Complete the Punnett square for the cross:

   (b) State the phenotype ratio of Manx to normal cats and explain why it is not the expected 3:1 ratio:

Gametes from female \ Gametes from male		

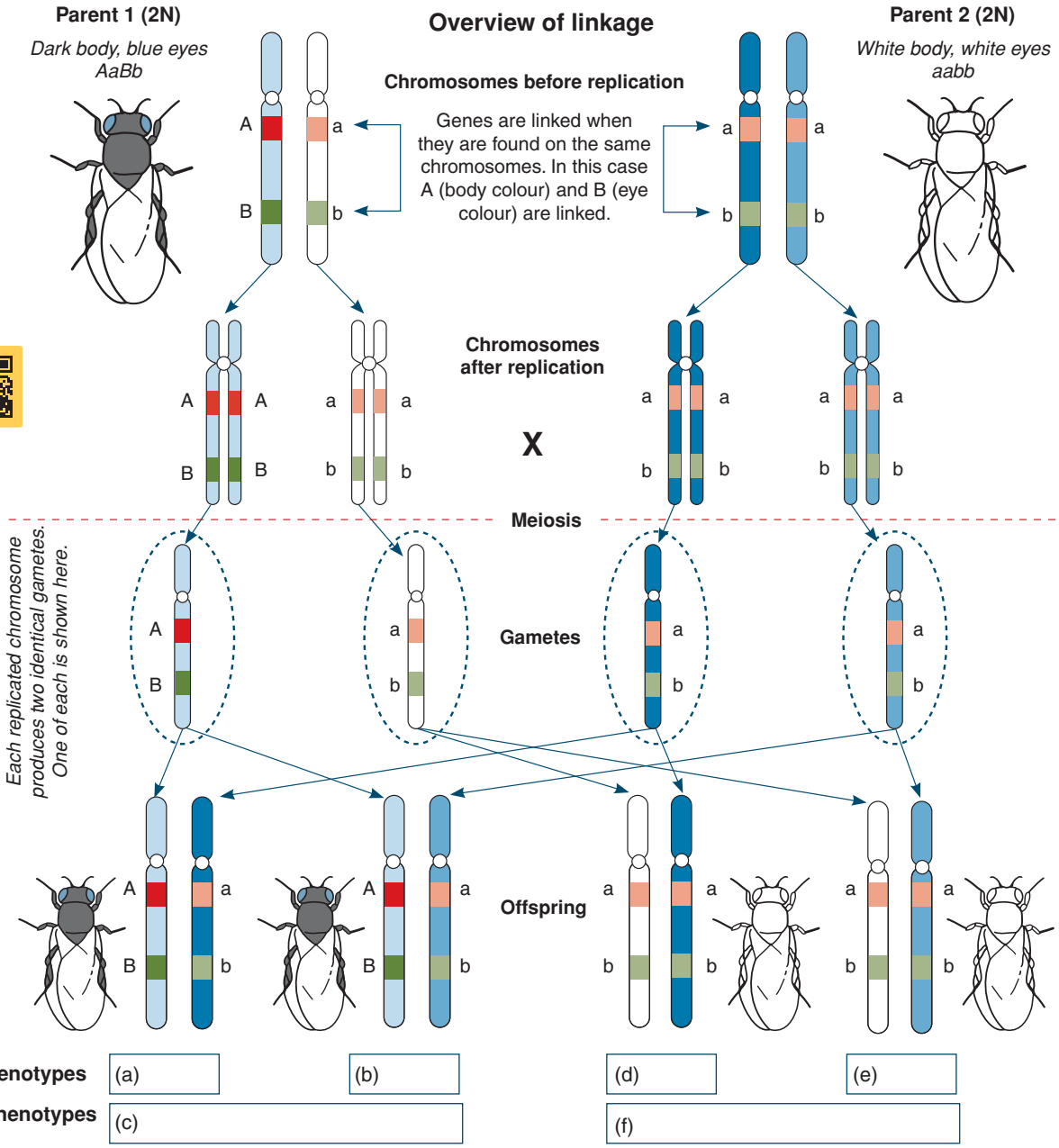
2. Huntington's disease (HD) is caused by an autosomal dominant mutation in either of the alleles of the gene, Huntingtin. Explain why HD persists in the human population, when it is caused by a lethal, dominant allele:

ISBN: 978-1-98-856655-9

85 Inheritance of Linked Genes

Key Idea: Linked genes tend to be inherited together. Linkage reduces the genetic variation in the offspring.

Genes are said to be linked when they are on the same chromosome. Linked genes tend to be inherited together. The likelihood of crossing over between linked genes decreases when genes are closer together. In genetic crosses, linkage is indicated when a greater proportion of the offspring are of the parental type than would be expected if the alleles were on separate chromosomes and assorting independently. Linkage reduces the genetic variation in the offspring.



Possible offspring

Only two kinds of genotype combinations are possible. They are the same as the parent genotype.

1. Complete the diagram above by writing the genotypes and phenotypes in the boxes provided:
2. What is the effect of linkage on the inheritance of genes? _____

_____

3. Explain how linkage decreases the amount of genetic variation in the offspring: _____

_____

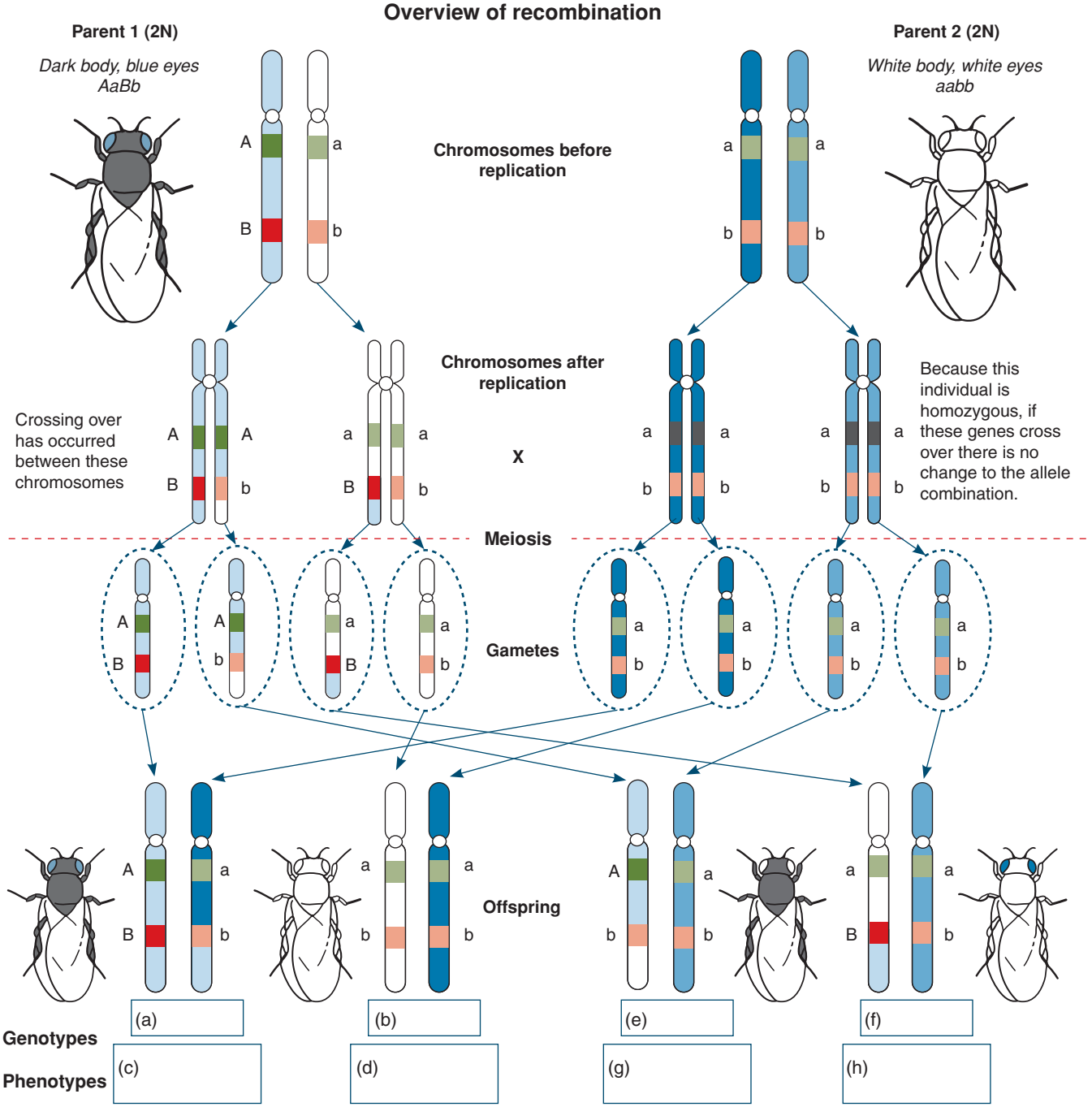
_____

ISBN: 978-1-98-856655-9

86 Recombination and Dihybrid Inheritance

Key Idea: Recombination is the exchange of alleles between homologous chromosomes as a result of crossing over. Recombination increases the genetic variation in the offspring. The alleles of parental linkage groups can separate in crossing over so that new associations of alleles are formed in the gametes (alleles are reshuffled). Offspring formed from these gametes show combinations of characteristics not seen in the parents and are called **recombinants**. In contrast to linkage, recombination increases genetic variation in the offspring. Recombination between the alleles of parental linkage groups is indicated by the appearance of non-parental types in the offspring

Overview of recombination



Non-recombinant offspring

These two offspring show allele combinations that are expected as a result of independent assortment during meiosis. Also called parental types.

Recombinant offspring

These two offspring show unexpected allele combinations. They can only arise if one of the parent's chromosomes has undergone crossing over.

1. Complete the diagram above by writing the genotypes and phenotypes in the boxes provided:

2. Describe the effect of recombination on the inheritance of genes: _____

_____

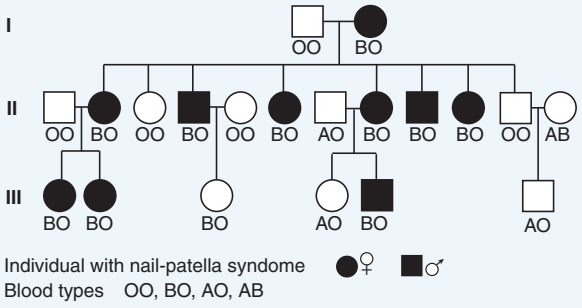
ISBN: 978-1-98-856655-9

87 Detecting Linkage in Dihybrid Crosses

Key Idea: Linkage affects the ratios of offspring.

Shortly after the rediscovery of Mendel's work early in the 20th century, it became apparent that his ratios of 9:3:3:1 for heterozygous dihybrid crosses did not always hold true. Experiments on sweet peas by William Bateson and Reginald Punnett, and on *Drosophila* by Thomas Hunt Morgan, showed evidence of some kind of coupling between many of the genes they studied. This coupling, which we now know to be linkage, did not follow any genetic relationship known at the time.

Pedigree for nail-patella syndrome



Linked genes can be detected by pedigree analysis (see Activity 92). The diagram above shows the pedigree for the inheritance of nail-patella syndrome, which results in small, poorly developed nails and kneecaps in affected people. The nail-patella syndrome gene is linked to the ABO blood group locus.

1. Fill in the missing numbers in the **expected** column of **Table 1**, remembering that a 9:3:3:1 ratio is expected:

2. (a) Fill in the missing numbers in the **expected** column of **Table 2**, remembering that a 1:1:1:1 ratio is expected:

   (b) Add the gamete type (parental/recombinant) to the gamete type column in Table 2:

   (c) What type of cross did Morgan perform here?

   _____

3. (a) Use the pedigree chart above to determine if nail-patella syndrome is dominant or recessive, giving reasons for your choice:

   _____

   _____

   _____

   _____

   (b) What evidence is there that nail-patella syndrome is linked to the ABO blood group locus?

   _____

   _____

   _____

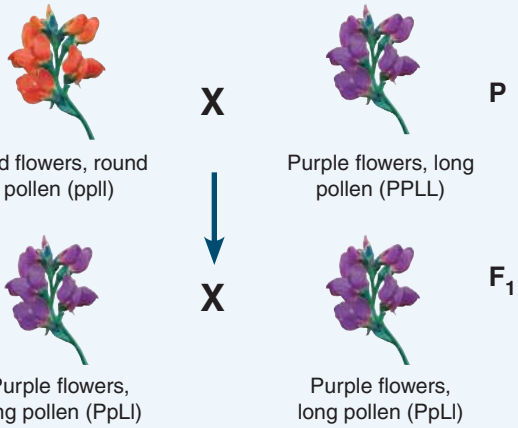
   (c) Suggest a likely reason why individual III-3 is not affected despite carrying the B allele:

   _____

   _____

   _____

Sweet pea cross



Bateson and Punnett studied sweet peas in which purple flowers (P) are dominant to red (p), and long pollen grains (L) are dominant to round (l). If these genes were unlinked, the outcome of a cross between two heterozygous sweet peas should have been a 9:3:3:1 phenotypic ratio.

Table 1: Sweet pea cross results

	Observed	Expected
Purple long (P_L_)	284	
Purple round (P_ll)	21	
Red long (ppL_)	21	
Red round (ppll)	55	
Total	381	381

Drosophila cross

Morgan performed experiments to investigate linked genes in *Drosophila*. He crossed a heterozygous red-eyed normal-winged (Prpr Vgvg) fly with a homozygous purple-eyed vestigial-winged (prpr vgvg) fly. The table (below) shows the outcome of the cross.

Table 2: *Drosophila* cross results

Genotype	Observed	Expected	Gamete type
Prpr Vgvg	1339		Parental
prpr Vgvg	152		
Prpr vgvg	154		
prpr vgvg	1195		
Total	2840	2840	

ISBN: 978-1-98-856655-9

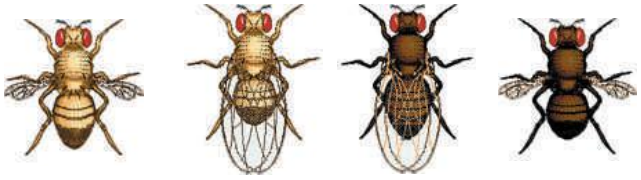
88 Testing the Outcomes of Genetic Crosses

Key Idea: A chi-squared (χ^2) test can be used to determine whether the outcome of a genetic cross is significantly different from the expected outcome.

When using the chi-squared test, the null hypothesis predicts the ratio of offspring of different phenotypes according to the expected Mendelian ratio for the cross, assuming independent assortment of alleles (no linkage). Significant departures from the predicted Mendelian ratio indicate linkage of the alleles in question. Raw counts should be used and a large sample size is required for the test to be valid.

Using χ^2 in Mendelian genetics

- In genetic crosses, certain ratios of offspring can be predicted based on the known genotypes of the parents. The chi-squared test is a statistical test used to determine how well observed offspring numbers match or fit expected numbers. Raw counts should be used and a large sample size is required for the test to be valid.
- In a chi-squared test, the null hypothesis (H_0) predicts that the ratio of offspring of different phenotypes is the same as the expected Mendelian ratio for the cross, assuming independent assortment of alleles (no linkage, i.e. the genes involved are on different chromosomes).
- Significant departures from the predicted Mendelian ratio indicate linkage (the genes are on the same chromosome) of the alleles in question.
- In a *Drosophila* genetics experiment, two individuals were crossed (the details of the cross are not relevant here). The predicted Mendelian ratios for the offspring of this cross were 1:1:1:1 for each of the four following phenotypes: grey body-long wing; grey body-vestigial wing; ebony body-long wing; ebony body-vestigial wing.
- The observed results of the cross were not exactly as predicted. The following numbers for each phenotype were observed in the offspring of the cross:



Grey body, vestigial wing	Grey body, long wing	Ebony body, long wing	Ebony body, vestigial wing
88	**98**	**102**	**112**

Table 1: Critical values of χ^2 at different levels of probability. By convention, the critical probability for rejecting the null hypothesis (H_0) is 5%. If the test statistic is less than the tabulated critical value for $P = 0.05$ we cannot reject H_0 and the result is not significant. If the statistic is greater than the tabulated value for $P = 0.05$ we reject (H_0) in favor of the alternative hypothesis.

Degrees of freedom	Level of probability (P)					
	0.50	**0.20**	**0.10**	**0.05**	**0.02**	**0.01**
1	0.455	1.64	2.71	3.84	5.41	6.64
2	1.386	3.22	4.61	5.99	7.82	9.21
3	2.366	4.64	6.25	7.82	9.84	11.35
4	3.357	5.99	7.78	9.49	11.67	13.28
5	4.351	7.29	9.24	11.07	13.39	15.09
	Do not reject H_0 ←				Reject H_0 →	

Steps in performing a χ^2 test for goodness of fit

1 Enter the observed value (O).

Enter the values of the offspring into the table (below) in the appropriate category (column 1).

2 Calculate the expected value (E).

In this case the expected ratio is 1:1:1:1. Therefore the number of offspring in each category should be the same (i.e. total offspring/ no. categories).
400 / 4 = 100 (column 2).

3 Calculate O – E and $(O-E)^2$

The difference between the observed and expected values is calculated as a measure of the deviation from a predicted result. Since some deviations are negative, they are all squared to give positive values (columns 3 and 4).

4 Calculate χ^2

For each category, calculate $(O - E)^2 / E$. Then sum these values to produce the χ^2 value (column 5).

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

5 Calculate degrees of freedom

The probability that any particular χ^2 value could be exceeded by chance depends on the number of degrees of freedom. This is simply one less than the total number of categories (this is the number that could vary independently without affecting the last value) In this case 4 – 1 = 3.

6 Use χ^2 table

On the χ^2 table with 3 degrees of freedom, the calculated χ^2 value corresponds to a probability between 0.2 and 0.5. By chance alone a χ^2 value of **2.96** will happen 20% to 50% of the time. The probability of 0.0 to 0.5 is higher than 0.05 (i.e 5% of the time) and therefore the null hypothesis cannot be rejected. We have no reason to believe the observed values differ significantly from the expected values.

	1	2	3	4	5
Category	O	E	O–E	$(O-E)^2$	$(O-E)^2/E$
GB, LW	98	100	-2	4	0.04
GB, VW	88	100	-12	144	1.44
EB, LW	102	100	2	4	0.04
EB, VW	112	100	12	144	1.44
				χ^2 →	2.96

ISBN: 978-1-98-856655-9

The following problems examine the use of the chi-squared (χ^2) test in genetics.

1. In a tomato plant experiment, two heterozygous individuals were crossed (the details of the cross are not relevant here). The predicted Mendelian ratios for the offspring of this cross were **9:3:3:1** for each of the **four following phenotypes**: purple stem-jagged leaf edge, purple stem-smooth leaf edge, green stem-jagged leaf edge, green stem-smooth leaf edge.

   The observed results of the cross were not exactly as predicted.
   The numbers of offspring with each phenotype are provided below:

Observed results of the tomato plant cross			
Purple stem-jagged leaf edge	**12**	Green stem-jagged leaf edge	**8**
Purple stem-smooth leaf edge	**9**	Green stem-smooth leaf edge	**0**

   (a) State your null hypothesis for this investigation (H_0): _____

   _____

   (b) State the alternative hypothesis (H_A): _____

   _____

2. Use the chi-squared (χ^2) test to determine if the differences between the observed and expected phenotypic ratios are significant. Use the table of critical values of χ^2 at different P values on the previous page.

   (a) Enter the observed values (number of individuals) and complete the table to calculate the χ^2 value:

Category	O	E	O — E	$(O - E)^2$	$\frac{(O - E)^2}{E}$
Purple stem, jagged leaf					
Purple stem, smooth leaf					
Green stem, jagged leaf					
Green stem, smooth leaf					
	Σ				Σ

   (b) Calculate χ^2 value using the equation:

   $$\chi^2 = \sum \frac{(O - E)^2}{E} \qquad \chi^2 = \_\_\_\_\_$$

   (c) Calculate the degrees of freedom: _____

   (d) Using the χ^2 table, state the *P* value corresponding to your calculated χ^2 value:

   _____

   (e) State your decision: *(circle one)*
   reject H_0 / do not reject H_0

3. Students carried out a pea plant experiment, where two heterozygous individuals were crossed. The predicted Mendelian ratios for the offspring were **9:3:3:1** for each of the **four following phenotypes**: round-yellow seed, round-green seed, wrinkled-yellow seed, wrinkled-green seed.

   The observed results were as follows:

Round-yellow seed	**441**	Wrinkled-yellow seed	**143**
Round-green seed	**159**	Wrinkled-green seed	**57**

   Use a separate piece of paper to complete the following:

   (a) State the null and alternative hypotheses (H_0 and H_A).

   (b) Calculate the χ^2 value.

   (c) Calculate the degrees of freedom and state the *P* value corresponding to your calculated χ^2 value.

   (d) State whether or not you reject your null hypothesis: reject H_0 / do not reject H_0 (circle one)

4. Comment on the whether the χ^2 values obtained above are similar. Suggest a reason for any difference:

   _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

89 Sex Linkage

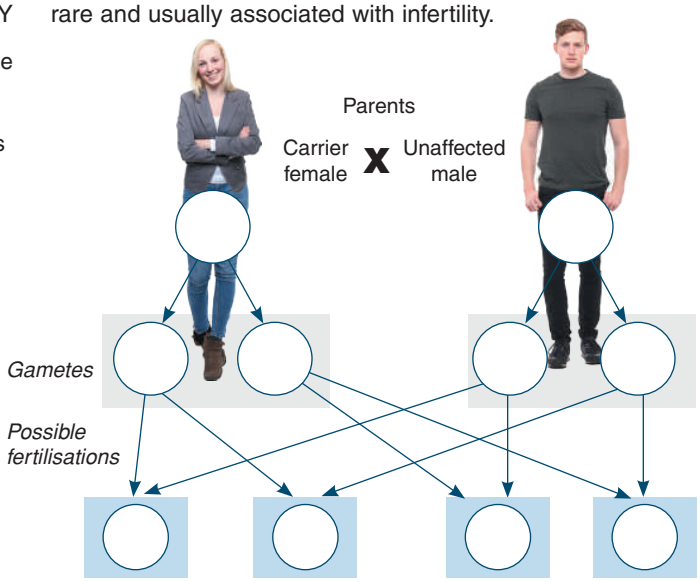
Key Idea: Many genes on the X chromosome do not have a match on the Y chromosome. In males, which are XY, a recessive allele on the X chromosome will be expressed.

Sex linkage refers to the way genes on the sex chromosomes are inherited and expressed. In humans, the sex chromosomes are X and Y, but sex linkage usually involves genes on the X chromosome, which has many more genes than the Y chromosome. X-linked recessive traits are usually seen only in males (XY) and occur rarely in the females (XX) because females may be heterozygous (carriers). X-linked dominant traits do not necessarily affect males more than females. In humans, recessive sex linked genes are responsible for a number of heritable disorders in males. Y-linked disorders are rare and usually associated with infertility.

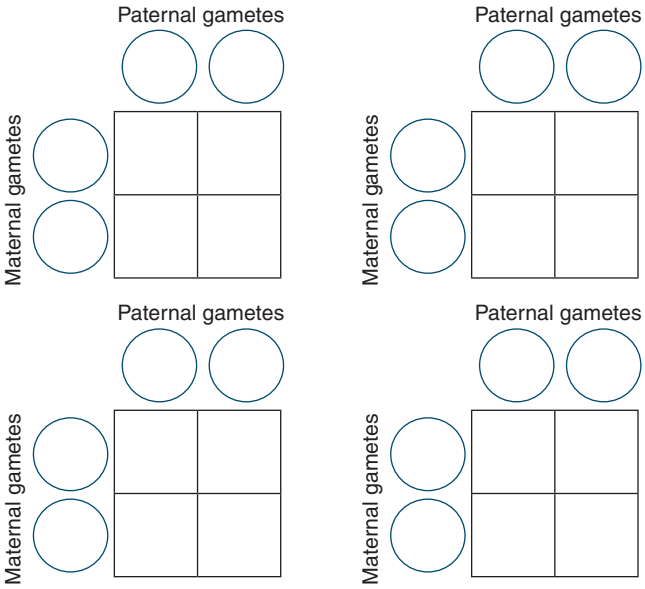
Haemophilia is a recessive disorder linked to the X-chromosome that results in ineffective blood clotting when a blood vessel is damaged. The most common type, haemophilia A, occurs in 1 in 5000 male births. Any male who carries the gene will express the phenotype. Haemophilia is extremely rare in women.

1. A couple wishes to have children. The woman knows she is a carrier for haemophilia. The man is not a haemophiliac. Use the notation X^h for haemophilia, X^H for the dominant allele, and Y for male to complete the diagram (right). Include the parent genotypes, gametes and possible fertilisations. Write the genotypes and phenotypes in the table below.

	Genotypes	Phenotypes
Male children		
Female children		



2. (a) A second couple also wishes to have children. The woman knows her maternal grandfather was a haemophiliac, but neither her mother or father were. Determine the probability of her being a carrier (X^HX^h). Use the Punnett squares (right) to help you:



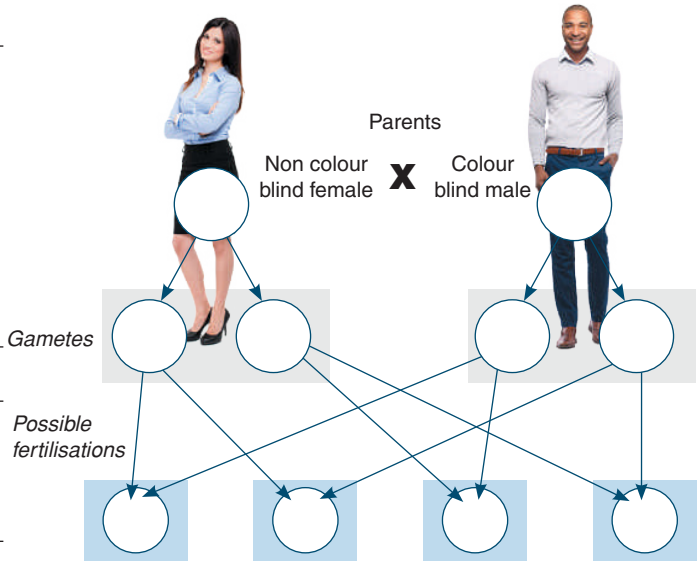
(b) The man is not a haemophiliac. Determine the probability that their first male child will have haemophilia. Use the Punnett squares to help you:

3. The gene for red-green colour vision is carried on the X chromosome. If the gene is faulty, colour blindness (X^b) will occur in males. Red-green colour blindness occurs in about 8% of males but in fewer than 1% of females. A colour blind man has children with a woman who is not colour blind. The couple have four children: 1 non colour blind son, 1 colour blind son, 2 non colour blind daughters. Describe the mother's:

(a) Genotype: _____

(b) Phenotype: _____

(c) Identify the genotype not possessed by any of the children:



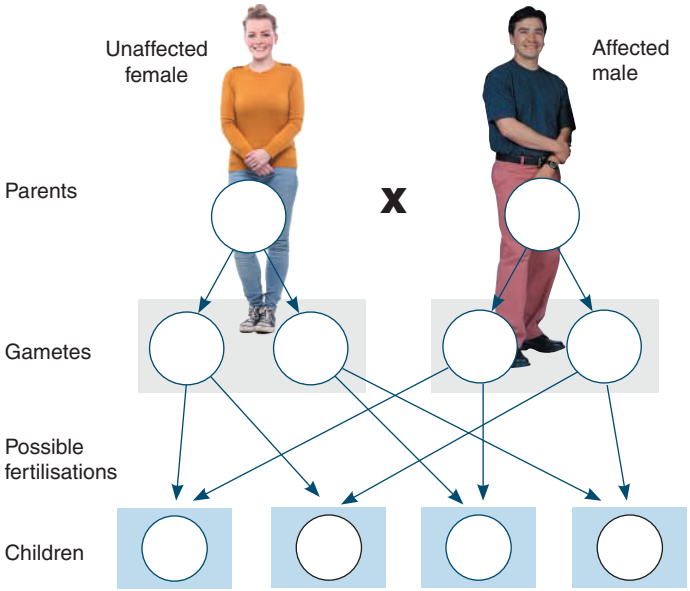
ISBN: 978-1-98-856655-9

Dominant allele in humans

A rare form of rickets in humans is determined by a dominant allele of a gene on the X chromosome. It is not found on the Y chromosome. This condition is not successfully treated with vitamin D therapy. The allele types, genotypes, and phenotypes are as follows:

Allele types	Genotypes	Phenotypes
X^R = affected by rickets	X^RX^R, X^RX =	Affected female
X = unaffected	X^RY =	Affected male
	XX, XY =	Unaffected female, unaffected male

As a genetic counsellor you are presented with a couple where one of them has a family history of this disease. The male is affected by this disease and the female is unaffected. The couple, who are thinking of starting a family, would like to know what their chances are of having a child born with this condition. They would also like to know what the probabilities are of having an affected boy or affected girl. Use the symbols above to complete the diagram (right) and determine the probabilities stated below (expressed as a proportion or percentage).



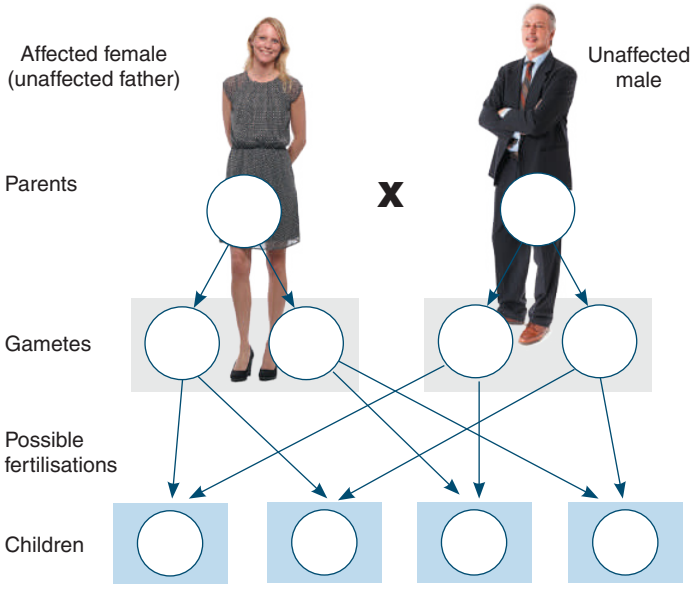
4. Determine the probability of having:

   (a) Affected children: _____

   (b) An affected girl: _____

   (c) An affected boy: _____

Another couple with a family history of the same disease also come in to see you to obtain genetic counselling. In this case, the male is unaffected and the female is affected. The female's father was not affected by this disease. Determine what their chances are of having a child born with this condition. They would also like to know what the probabilities are of having an affected boy or affected girl. Use the symbols above to complete the diagram (right) and determine the probabilities stated below (expressed as a proportion or percentage).



5. Determine the probability of having:

   (a) Affected children: _____

   (b) An affected girl: _____

   (c) An affected boy: _____

6. Why are males much more likely to inherit X-linked recessive disorders than females?

_____

_____

_____

7. From what you know about sex linkage, what two features could you use to detect a Y-linked disorder in a pedigree?

   (a) _____

   (b) _____

ISBN: 978-1-98-856655-9

90 Predicting Genetic Outcomes

Key Idea: For monohybrid and dihybrid crosses the offspring appear in predictable ratios.

Test your understanding of monohybrid crosses by solving these problems involving the inheritance of a single gene.

1. A dominant gene (W) produces wire-haired texture in dogs; its recessive allele (w) produces smooth hair. A group of heterozygous wire-haired individuals are crossed and their progeny are then **test-crossed**. Determine the expected genotypic and phenotypic ratios among the test cross progeny:

2. In sheep, black wool is due to a recessive allele (b) and white wool to its dominant allele (B). A white ram is crossed to a white ewe. Both animals carry the black allele (b). They produce a white ram lamb, which is then **back crossed** to the female parent. Determine the probability of the back cross offspring being black:

3. A recessive allele, **a**, is responsible for albinism, an inability to produce or deposit melanin in tissues. Humans and a variety of other animals can exhibit this phenotype. In each of the following cases, determine the possible genotypes of the mother and father, and of their children:

   (a) Both parents have normal phenotypes; some of their children are albino and others are unaffected:

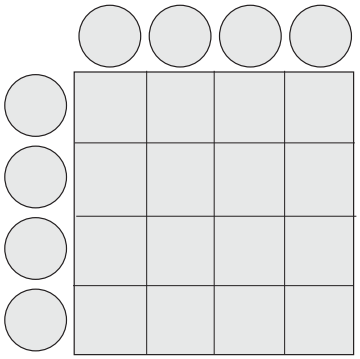
   (b) Both parents are albino and have only albino children:

   (c) The woman is unaffected, the man is albino, and they have one albino child and three unaffected children:

4. Two mothers give birth to sons at a busy hospital. The son of the first couple has haemophilia, a recessive, X-linked disease. Neither parent from couple #1 has the disease. The second couple has an unaffected son, despite the fact that the father has haemophilia. The two couples challenge the hospital in court, claiming their babies must have been swapped at birth. You must advise as to whether or not the sons could have been swapped. What would you say?

ISBN: 978-1-98-856655-9

5. In rabbits, spotted coat S is dominant to solid colour s, while for coat colour, black B is dominant to brown b. A brown spotted rabbit is mated with a solid black one and all the offspring are black spotted (the genes are not linked).



(a) State the genotypes:

Parent 1: _____

Parent 2: _____

Offspring: _____

(b) Use the Punnett square to show the outcome of a cross between the F_1 (the F_2):

(c) Using ratios, state the phenotypes of the F_2 generation: _____

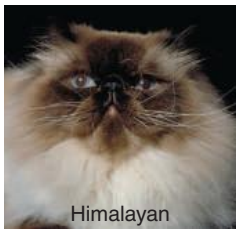
_____

_____

6. The Himalayan colour-pointed, long-haired cat is a breed developed by crossing a pedigree (true-breeding), uniform-coloured, long-haired Persian with a pedigree colour-pointed (darker face, ears, paws, and tail) short-haired Siamese.


Persian


Siamese

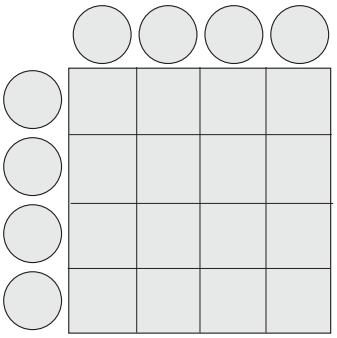

Himalayan

The genes controlling hair colouring and length are on separate chromosomes: uniform colour U, colour pointed u, short hair S, long hair s.

(a) Using the symbols above, indicate the genotype of each breed below its photograph (above, right): _____ _____ _____

(b) State the genotype of the F_1 (Siamese X Persian): _____

(c) State the phenotype of the F_1: _____



(d) Use the Punnett square to show the outcome of a cross between the F_1 (the F_2):

(e) State the ratio of the F_2 that would be Himalayan: _____

(f) State whether the Himalayan would be true breeding: _____

(g) State the ratio of the F_2 that would be colour-point, short-haired cats: _____

7. A Drosophila male with genotype CucuEbeb (straight wing, grey body) is crossed with a female with genotype cucuebeb (curled wing, ebony body). The phenotypes of the F1 were recorded and the percentage of each type calculated. The percentages were: Straight wings, grey body 45%, curled wings, ebony body 43%, straight wings, ebony body 6%, and curled wings grey body 6%.

(a) Is there evidence of crossing over in the offspring? _____

(b) Explain your answer: _____

_____

_____

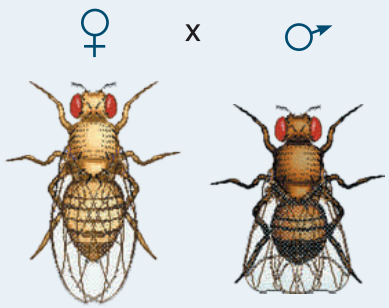
_____

_____

(c) Determine the genotypes of the offspring: _____

_____

_____



Straight wing Cucu Grey body, Ebeb

Curled wing cucu Ebony body, ebeb

8. For the cross TTgg x TtGg with unlinked genes, how many different genotypes and phenotypes are expected?

_____

ISBN: 978-1-98-856655-9

91 Inheritance Patterns

Key Idea: Sex-linked traits and autosomal traits have different inheritance patterns.

Complete the following monohybrid crosses for different types of inheritance patterns in humans: autosomal recessive, autosomal dominant, sex linked recessive, and sex linked dominant inheritance.

1. **Inheritance of autosomal recessive traits**
   Example: *Albinism*
   Albinism (lack of pigment in hair, eyes and skin) is inherited as an autosomal recessive allele (not sex-linked).
   Using the codes: **PP** (not albino) **Pp** (carrier)
   pp (albino)

   (a) Enter the parent phenotypes and complete the Punnett square for a cross between two carrier genotypes.
   (b) Give the ratios for the phenotypes from this cross.

   Phenotype ratios: _____

   _____

2. **Inheritance of autosomal dominant traits**
   Example: *Woolly hair*
   Woolly hair is inherited as an autosomal dominant allele. Each affected individual will have at least one affected parent.
   Using the codes: **WW** (woolly hair)
   Ww (woolly hair, heterozygous)
   ww (hair not woolly)

   (a) Enter the parent phenotypes and complete the Punnett square for a cross between two heterozygous individuals.
   (b) Give the ratios for the phenotypes from this cross.

   Phenotype ratios: _____

   _____

3. **Inheritance of sex linked recessive traits**
   Example: *Haemophilia*
   Inheritance of haemophilia is sex linked. Males with the recessive (haemophilia) allele, are affected. Females can be carriers.
   Using the codes: **XX** (unaffected female)
   X^hX (carrier female)
   X^hX^h (haemophiliac female)
   XY (unaffected male)
   X^hY (haemophiliac male)

   (a) Enter the parent phenotypes and complete the Punnett square for a cross between an unaffected male and a carrier female.
   (b) Give the ratios for the phenotypes from this cross.

   Phenotype ratios: _____

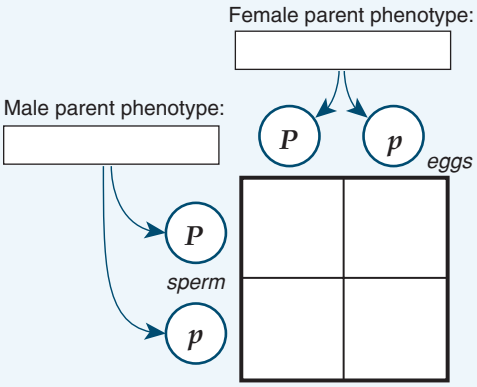
   _____

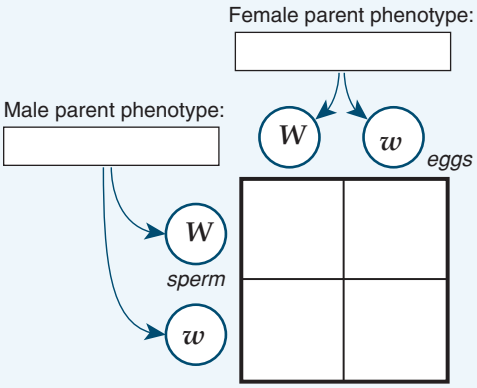
4. **Inheritance of sex linked dominant traits**
   Example: *Sex linked form of rickets*
   A rare form of rickets is inherited on the X chromosome.
   Using the codes: **XX** (unaffected female); XY (unaffected male)
   X^RX (affected heterozygote female)
   X^RX^R (affected female)
   X^RY (affected male)

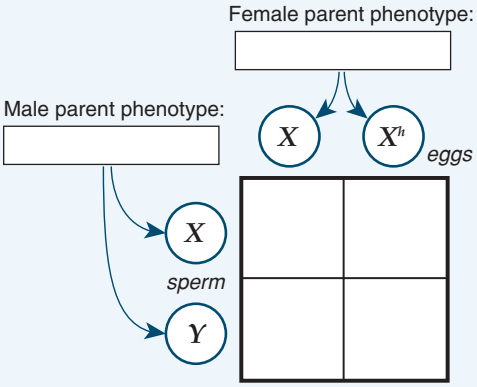
   (a) Enter the parent phenotypes and complete the Punnett square for a cross between an affected male and heterozygous female.
   (b) Give the ratios for the phenotypes from this cross.

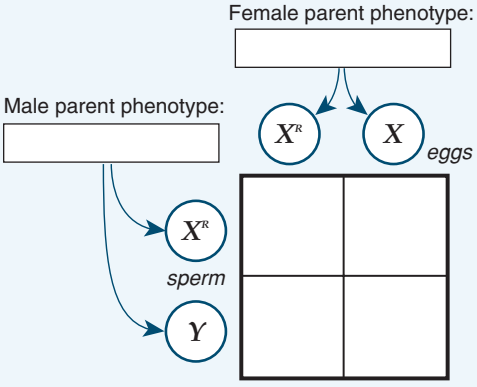
   Phenotype ratios: _____

   _____









ISBN: 978-1-98-856655-9

92 Pedigree Analysis

Key Idea: Pedigree charts are a way to graphically illustrate inheritance patterns over a number of generations.

One way in which to analyse the family history of an observable trait is to use a pedigree chart, which follows certain rules and uses particular symbols to indicate the sex and genotype of individuals across generations.

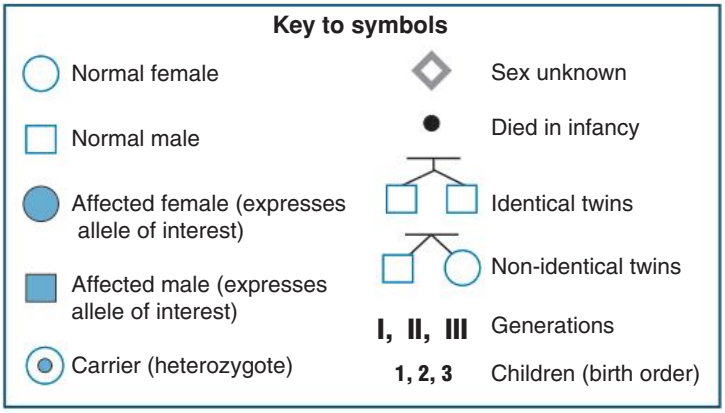
Pedigree charts

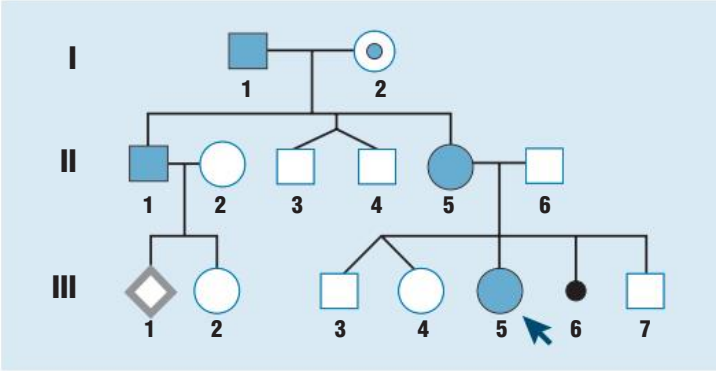
A pedigree chart is a diagram that shows the occurrence of a particular gene or trait from one generation to the next. In humans, pedigree charts are often used to analyse the inheritance of heritable conditions. In domestic animals, pedigree charts are often used to trace the inheritance of traits in selective breeding programs.

Pedigree charts use symbols to indicate an individual's particular traits. The key (right) explains the meaning of the symbols. Particular individuals are identified by their generation number and their order in that generational row. For example, **II-6** is the sixth person in the second generation. The arrow indicates the person through whom the pedigree was discovered (i.e. reported the condition).

The chart on the right represents three generations: grandparents (I-1 and I-2) with three sons and one daughter. Two of the sons (II-3 and II-4) are identical twins, but did not have any children. The other son (II-1) had a daughter and another child (sex unknown). The daughter (II-5) had two sons and two daughters, plus a child that died in infancy. Pedigrees can also indicate if a trait shows autosomal or sex linked inheritance. In autosomal patterns, both males and females are generally equally affected (more or less).

For the particular trait being studied, the grandfather was expressing the phenotype (showing the trait) and the grandmother was a carrier. One of their sons and one of their daughters also show the trait, together with one of their granddaughters (arrow).





1. (a) Brown eyes are the result of a dominant eye-colour allele and blue eyes are recessive. A brown-eyed man (A), whose mother had blue eyes and whose father had brown eyes, marries a blue-eyed woman (B) whose parents are both brown-eyed. They have a daughter who is blue-eyed. Draw a pedigree showing all four grandparents, the two parents, and the daughter. Indicate each individual's possible genotype. Use filled shapes to indicate the recessive trait.

(b) Identify the individuals that are definitely heterozygous (carriers): _____

(c) Identify the individual that could be heterozygous (a carrier): _____

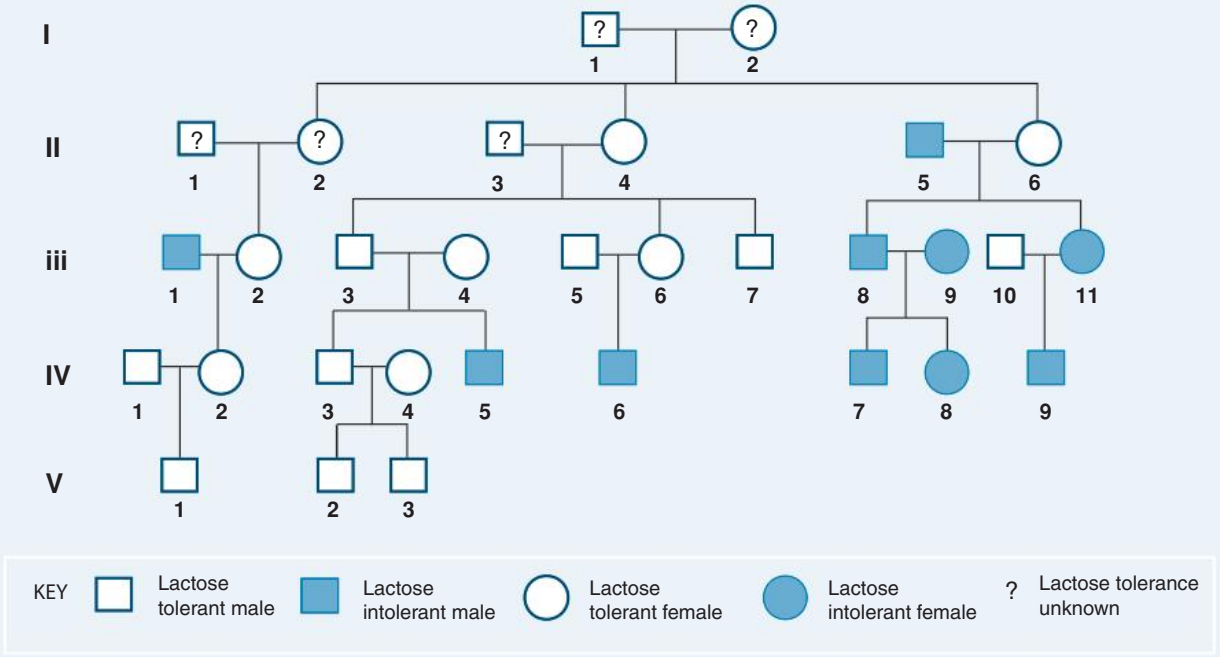
(d) What is the probability of couple A and B having a blue-eyed boy as their next child? _____

(e) Explain your reasoning: _____

ISBN: 978-1-98-856655-9

The pedigree of lactose intolerance

Lactose intolerance is the inability to digest the milk sugar lactose. It occurs because some people do not produce lactase, the enzyme needed to break down lactose. The pedigree chart below was one of the original studies to determine the inheritance pattern of lactose intolerance.



2. Use an analysis of the pedigree above to make a claim about the inheritance pattern of lactose intolerance. Support your claim with at least two pieces of evidence:

3. (a) Use the Punnett square below to show the cross between III-10 and III-11 in the pedigree chart above. Use the capital letter L for the dominant allele and the letter l for the recessive allele.

   (b) Explain how you can be certain about III-10's genotype:

	Male alleles		
Female alleles			

4. What is the probability that V-1 is heterozygous for lactose intolerance (LI)? Show your working or justification:

5. How do we know that the original parents (row I) could not both have been homozygous dominant? Explain:

ISBN: 978-1-98-856655-9

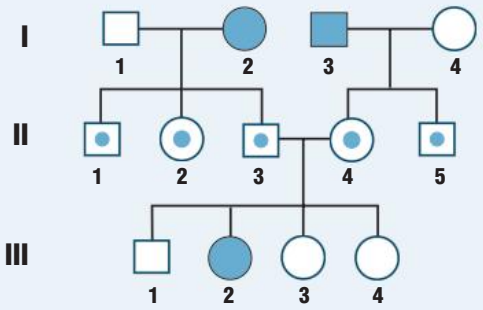
6. **Autosomal recessive traits**

Albinos lack pigment in the hair, skin and eyes. This is an autosomal recessive trait.

(a) Write the genotype for each of the individuals on the chart using the following letter codes: **PP** normal skin colour; **P-** normal, but unknown if homozygous; **Pp** carrier; **pp** albino.

(b) Why must the parents (II-3) and (II-4) be carriers of a recessive allele?

Albinism in humans



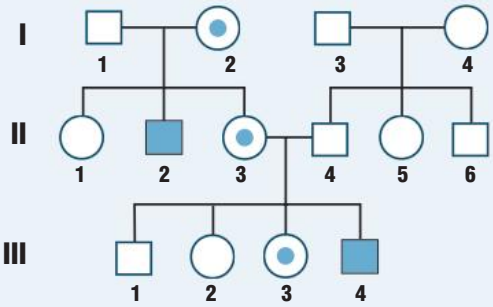
7. **Sex linked recessive traits**

Haemophilia is a disease where blood clotting is affected. A person can die from a simple bruise (which is internal bleeding). The clotting factor gene is carried on the X chromosome.

(a) Write the genotype for each of the individuals on the chart using the codes: **XY** normal male; **X_hY** affected male; **XX** normal female; **X_hX** female carrier; **X_hX_h** affected female:

(b) Why can males never be carriers?

Haemophilia in humans



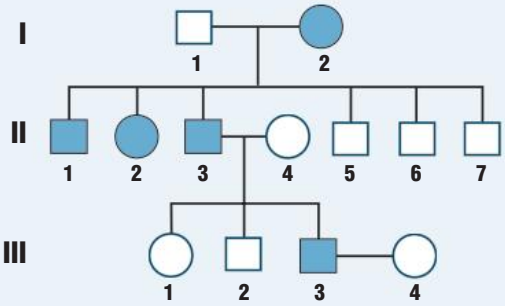
8. **Autosomal dominant traits**

An unusual trait found in some humans is woolly hair (not to be confused with curly hair). Each affected individual will have at least one affected parent.

(a) Write the genotype for each of the individuals on the chart using the following letter codes:
WW woolly hair; **Ww** woolly hair (heterozygous); **W-** woolly hair, but unknown if homozygous; **ww** normal hair

(b) Describe a feature of this inheritance pattern that suggests the trait is the result of a **dominant** allele:

Woolly hair in humans



9. **Sex linked dominant traits**

A rare form of rickets is inherited on the X chromosome. All daughters of affected males will be affected. More females than males will show the trait.

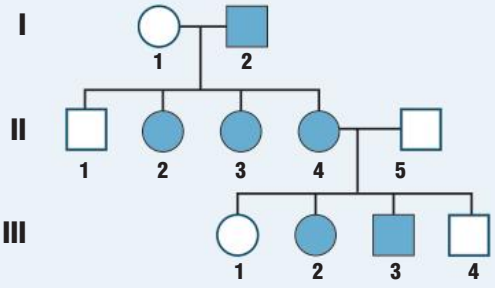
(a) Write the genotype for each of the individuals on the chart using the following letter codes:
XY normal male; **X_RY** affected male; **XX** normal female; **X_{R-}** female (unknown if homozygous); **X_RX_R** affected female.

(b) What is the probability a male offspring will be affected if either of the parents (one or the other) are affected?

(c) What is the probability a female offspring will be affected if either of the parents (one of the other) are affected?

(d) Why will more females than males be affected?

A rare form of rickets in humans



ISBN: 978-1-98-856655-9

93 Analysing Allele Frequencies

Key Idea: The frequency in which alleles appear in different populations varies. Allele frequencies can be used in many genetic studies and forensic tests.

Recall that an allele is a version of a gene. Analysing how often an allele occurs relative to the total number of alleles (the allele frequency) can be useful in population studies. Recording how allele frequencies change over time can give insight in the evolution of a population. Comparing allele frequencies between populations can show important differences between populations.

Calculating allele frequency

- The frequency of an allele represents how often an allele appears in a gene pool or population. The frequency is calculated by dividing the number of times the allele is observed by the total number of alleles for that gene or genetic locus.

$$\text{Allele frequency} = \frac{\text{Number of copies of a specific allele}}{\text{Total number of alleles in population}}$$

- For example, in Awassi sheep (right), a mutation in the gene CNGA3 produces an allele that, in homozygous recessive animals, causes day blindness. In a population of 423 sheep 74 were found to be carriers (heterozygous for the mutation). Therefore the frequency of the mutant allele is 74 ÷ (423 x 2) = 0.087

Allele frequencies and gene pools

- Recording changes in gene pools can give insight into the evolution of a population. In Olney, Illinois, there is a unique population of albino (white) and grey squirrels. Between 1977 and 1990, students at Olney Central College carried out a study of this population. They recorded the frequency of grey and albino squirrels. The albinos displayed a mutant allele expressed as an albino phenotype only in the homozygous recessive condition. The recorded results are shown below:

Population of grey and white squirrels in Olney, Illinois (1977-1990)

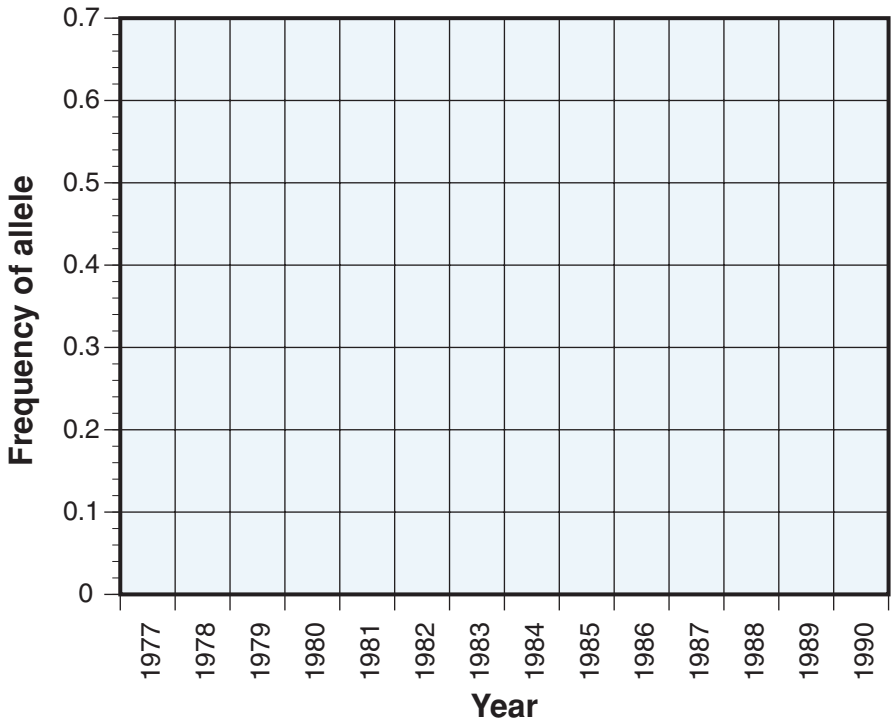
Year	Grey (GG)	Grey (Gg)	White (gg)	Total	Freq. of G	Freq. of g
1977	211	391	182	784	0.48	0.52
1978	170	342	172	683		
1979	175	307	134	616		
1980	180	309	133	622		
1981	187	349	163	699		
1982	238	380	151	769		
1983	139	280	141	560		
1984	137	241	106	484		
1985	163	285	125	573		
1986	191	345	155	691		
1987	*No data collected this year*					
1988	281	371	122	774		
1989	206	346	146	698		
1990	262	341	111	714		

1. Explain the equation for calculating the frequency of the mutant allele in Awassi sheep: _____

2. Complete the table above by calculating the frequency of the G and g alleles in the squirrel population:

ISBN: 978-1-98-856655-9

3. Plot a graph of the frequency of the G and g alleles over time:



4. Describe the shape of the graph and what this means for the evolution of the squirrel's gene pool:

_____

_____

_____

Allele frequencies and SNPs

- Single nucleotide polymorphisms (SNPs, pronounced 'snips') are the most common genetic variation among people. A SNP is a single nucleotide change at a specific DNA locus, normally found in at least 1% of the population. For example a sequence of DNA may have two polymorphisms: AAC**G**TGT or AAC**T**TGT.
- SNPs are mostly found in between genes and therefore have little effect on gene function. However, a particular SNP or set of SNPs may be associated with a gene or, more specifically, with an allele of a gene. This means they are close enough to the gene that they are inherited with it. As a result, a SNP can be used as a gene marker, i.e. a specific SNP identifies a specific allele.
- Large numbers of SNPs have been identified in humans. Specific groups of SNPs are called haplotypes and specific haplotypes may be associated with specific diseases, or how people react to certain medication.
- Identifying haplotypes gives scientists a way of identifying the genetic cause of disease or to devise better treatments for a person's illness.

5. What is a SNP? _____

6. Explain why SNP markers need to be close to they respective gene: _____

_____

_____

7. Why would identifying haplotypes associated with how a person reacts to treatment help scientists device better treatments?

_____

_____

_____

ISBN: 978-1-98-856655-9

94 Quantitative Investigation of Variation

Key Idea: Differences between two populations can be tested for significance using the Student's *t* test.

The purpose of selective breeding is to produce a phenotypic change in the next generation, e.g. longer leaves. The significance of this change can be tested using the Student's *t* test. This is commonly used to compare two sample means, e.g. means for a measured characteristic between two populations. It is a simple test for distinguishing differences between samples and is robust even when sample sizes are small. An example outlining the steps in the test is given below. It compares data for a treatment and a control from a hypothetical experiment (units are not relevant in this case).

Steps in performing a Student's *t* test

1 Calculate summary statistics for the two data sets

Control (A)	Treatment (B)
6.6	6.3
5.5	7.2
6.8	6.5
5.8	7.1
6.1	7.5
5.9	7.3

$n_A = 6$, $\bar{x}_A = 6.12$, $s_A = 0.496$

$n_B = 6$, $\bar{x}_B = 6.98$, $s_B = 0.475$

n_A and n_B are the number of values in the first and second data sets respectively (these do not need to be the same).

$\bar{x}$ is the mean.

s is the standard deviation (a measure of scatter in the data).

2 Set up and state your null hypothesis (H_0)

H_0: there is no treatment effect. The differences in the data sets are the result of chance and they are not really different. The alternative hypothesis is that there is a treatment effect and the two sets of data are truly different.

3 Decide if your test is one or two tailed

A one-tailed test looks for a difference only in one particular direction. A two-tailed test looks for any difference (+ or –). This tells you what section of the t table to consult. Most biological tests are two-tailed. Very few are one-tailed.

4 Calculate the t statistic

For our sample data above the calculated value of t is –3.09. The degrees of freedom (df) are $n_1 + n_2 - 2 = 10$.

Calculation of the *t* value uses the variance which is simply the square of the standard deviation (s^2). You may compute *t* using a spreadsheet but manual computation is not difficult (see opposite). It does not matter if the calculated *t* value is a positive or negative (the sign is irrelevant).

The absolute value of the *t* statistic (3.09) well exceeds the critical value for $P = 0.05$ at 10 degrees of freedom.

We can reject H_0 and conclude that the means are different at the 5% level of significance.

If the calculated absolute value of *t* had been less than 2.23, we could not have rejected H_0.

1. (a) In an experiment, data values were obtained from four plants in experimental conditions and three plants in control conditions. The mean values for each data set (control and experimental conditions) were calculated. The *t* value was calculated to be 2.16. The null hypothesis was: 'The plants in the control and experimental conditions are not different'. State whether the calculated *t* value supports the null hypothesis or its alternative (consult *t* table below):

   (b) The experiment was repeated, but this time using 6 control and 6 'experimental' plants. The new *t* value was 2.54. State whether the calculated *t* value supports the null hypothesis or its alternative now:

2. Explain what you understand by statistical significance:

Table of critical values of *t* at different levels of *P*.

Degrees of freedom	Level of Probability		
	0.05	0.01	0.001
1	12.71	63.66	636.6
2	4.303	9.925	31.60
3	3.182	5.841	12.92
4	2.776	4.604	8.610
5	2.571	4.032	6.869
6	2.447	3.707	5.959
7	2.365	3.499	5.408
8	2.306	3.355	5.041
9	2.262	3.250	4.781
10	2.228	3.169	4.587
15	2.131	2.947	4.073
16	2.120	2.921	4.015
17	2.110	2.898	3.965
18	2.101	2.878	3.922
19	2.093	2.861	3.883
20	2.086	2.845	3.850
25	2.060	2.787	3.725
30	2.042	2.750	3.646
40	2.021	2.704	3.551
50	2.009	2.678	3.496
100	1.984	2.626	3.390
200	1.972	2.601	3.340

ISBN: 978-1-98-856655-9

Quantifying differences between generations

The effect of selective breeding can be studied using Wisconsin Fast Plants®, plants bred to complete their life cycle in only five weeks. These plants show variation in quantifiable traits such hairiness (number of trichomes) and stem colour. Students conducted a selective breeding experiment and recorded the results below.

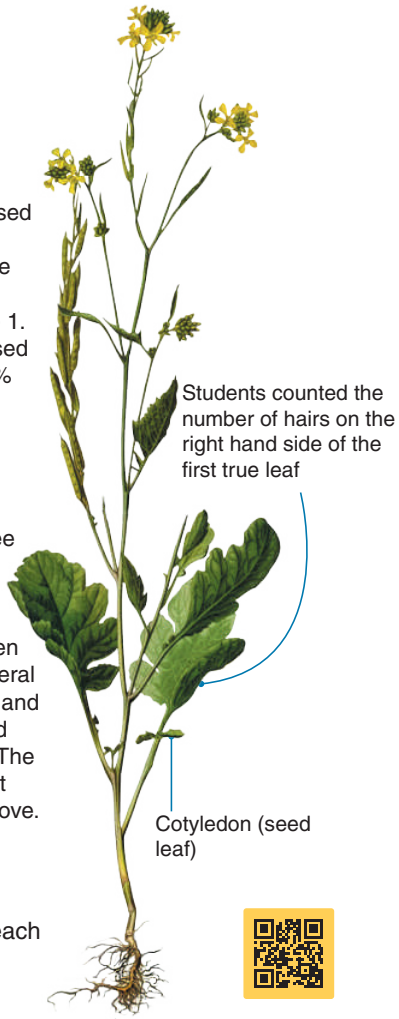
The students chose hairiness (number of trichomes) and selected plants by cross-pollinating the hairiest plants within a parental generation to produce a generation of offspring (F_1). The incidence of hairiness in the F_1 generation was studied to quantify the effect of artificial selection on phenotype.

Procedure

Students planted and grew Wisconsin Fast Plants® *Brassica rapa* seeds in the laboratory taking care to cultivate them in the soil, light, and moisture conditions required for optimal growth. At maturity (7-12 days) the students used a magnifier to count the number of trichomes (hairs) on the edge of the right hand side of the first true leaf of each plant. The class data for the parental generation are presented in Table 1. From the parental generation (F_0), students used small tags to identify and label the hairiest 10% of plants.

At day 14, when several flowers were present on each plant, the students cross pollinated the hairiest 10% of plants using pollination wands. This procedure was carried out for three consecutive days to ensure pollination was successful and fertilisation had occurred.

Seeds were harvested from each plant between days 28-36, and placed in a paper bag for several days to dry. Once dry, the seeds were planted and grown under the same conditions as described above to produce the F_1 generation of plants. The number of hairs on each plant were counted at maturity using the same method described above. The results are presented in Table 1.



3. Record the frequency of trichomes for each of the categories listed below in table 2:

Table 2.
Frequency of trichomes in parental and first generation plants

Number of trichomes	Parental generation		F_1 generation	
	Working	Frequency	Working	Frequency
0-4	46+6+3+3+2			
5-9				
10-14				
15-19				
20-24				
25-29				
30-34				
35-39				
40-44				

Table 1. Trichome data for parental and F_1 generation fast plants

Number of trichomes	Parental generation		F_1 generation	
	Freq.	Total	Freq.	Total
0	46	0	8	0
1	6		3	
2	3		1	
3	3		0	
4	2		1	
5	6		1	
6	4		3	
7	2		4	
8	7		2	
9	2		3	
10	7		4	
11	3		4	
12	6		2	
13	0		5	
14	1		2	
15	2		6	
16	3		1	
17	1		7	
18	2		3	
19	1		5	
20	1		8	
21	3		1	
22	2		0	
23	0		3	
24	1		1	
25	0		1	
26	1		2	
27	0		0	
28	0		2	
29	1		2	
30	0		1	
31	0		2	
32	2		1	
33	1		0	
34	0		0	
35	0		1	
36	0		0	
37	0		0	
38	0		0	
39	0		0	
40	0		1	
41	0		0	
42	0		0	
43	0		0	
44	1		0	
Totals	120		91	

ISBN: 978-1-98-856655-9

4. (a) Select an appropriate graph type and plot trichome distribution in the parental and F_1 generation plants above:

   (b) Describe the distribution of trichomes between the two sets: _____

5. (a) Calculate the total number of trichomes in the totals columns of Table 1.

   (b) From the data, how would you calculate the mean number of trichomes on the right-hand side of the first true leaf?

   (c) How could you test if any difference was significant? _____

   (d) How could you further explore the heritability of trichome density under selection? _____

ISBN: 978-1-98-856655-9

Testing for significance

- A Student's *t* test to can be used to determine if the F_1 generation is significantly hairier that the F_0 generation. To do this you will need to use a spreadsheet:

Investigation 4.1 Investigating phenotypic difference

See appendix for equipment list.

1. Open a new spreadsheet.

2. In **cell B1** type the heading: No. of trichomes F_0. In **cell C1** type the heading: Frequency of trichomes F_0.

3. Fill **column B** down to the number 44, starting at 0 (as table 1 on the previous page).

	B	C
1	No. of trichomes	Freq of trichomes on leaf F0
2	0	46
3	1	6
4	2	3

4. Starting in **cell C2** enter the data from the second column of table 1 on the previous page (frequency). You should now have two columns of data as per the first two columns from table 1.

5. The data represents the number of times each number of trichomes was recorded. For example, 46 leaves had 0 trichomes, 6 leaves had 5 trichomes and so on. The data must be spread back out into its original record. This can be done using the VLOOKUP formula.

6. First type the heading: **help 1** into **cell A1**. In **cell A2** type the number 1.

7. In **cell A3** type the formula: **=A2+C2**. Select **cell A3** and fill down to **cell A47** (which should show the number 121).

8. In **cell D1** type the heading: **help 2** from cell D2 fill down to cell D121 (which should show 120).

	A	D	E
1	help 1	help 2	Original data
2	1	1	=VLOOKUP(D2,$A$1:$B$46,2)
3	=A2+C2	2	=VLOOKUP(D3,$A$1:$B$47,2)
4	=A3+C3	3	=VLOOKUP(D4,$A$1:$B$47,2)

9. You are now ready to create the VLOOKUP table and spread out the tallied data. In **cell E1** type the heading: **Original data**.

10. In **cell E2** type the formula: **=VLOOKUP(D2,$A$1:$D$46,2)**. Copy this formula down to **cell E121**.

11. In **cell D123** type the heading: Mean. In **cell E123** type the formula **=AVERAGE(E2:E121)**.

	E
122	
123	=AVERAGE(E2:E121)
124	
125	=STDEV(E2:E121)

12. In **cell D125** type the heading: Standard deviation A. In **cell E125** type the formula **=STDEV(E2:E121)**.

13. Repeat steps 2–12 for the F1 generation in the table on the previous page, starting at **cell H1** instead of A1. You will need to adjust the formula accordingly. Remember that the F_1 generation tested a total of 91 plants, rather than 120.

14. Once you have both sets of data entered you can carry out the Student's t test.

15. In **cell A51** type the heading: t value. In cell **B51** you need a formula that will return the t value to look up in the table of critical values of t. The linear formula for calculating a t value is $t = (\bar{x}_a - \bar{x}_b) \div s((s^2_a/n_a) + (s^2_b/n_b))$ where $\bar{x}_a$ and $\bar{x}_b$ are the means, s_a and s_b are the standard deviations, and n_a and n_b are the population numbers. After you have calculated these values in your spreadsheet you can substitute them into the formula to use in your spreadsheet (or use the cell numbers). The general formula is: **$=(\bar{x}_a-\bar{x}_b)/SQRT((s_a\text{^}2/120)+(s_b\text{^}2/91))$**.

	E
128	=(E123-L94)/SQRT((E125^2/120)+(L96^2/91))
129	

16. You can compare the number returned with the t table on the previous page. Remember degrees of freedom is $n_1 + n_2 - 2$.

6. (a) State the mean and standard deviation of trichomes for the F_0 generation: _____

   (b) State the mean and standard deviation of trichomes for the F1 generation: _____

7. (a) State the t value you calculated: _____

   (b) Is the hairiness between the generations significantly different at the P = 0.05 level? _____

ISBN: 978-1-98-856655-9

95 Chapter Review: Did You Get It?

1. Study the pedigree chart on the right.

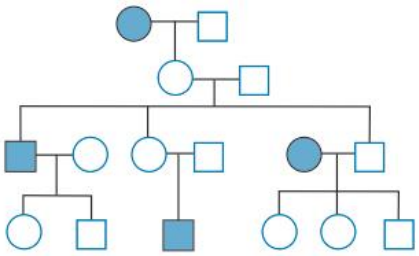
   (a) What type of inheritance pattern is shown? _____

   (b) Give a reason for your answer: _____

   _____

   _____

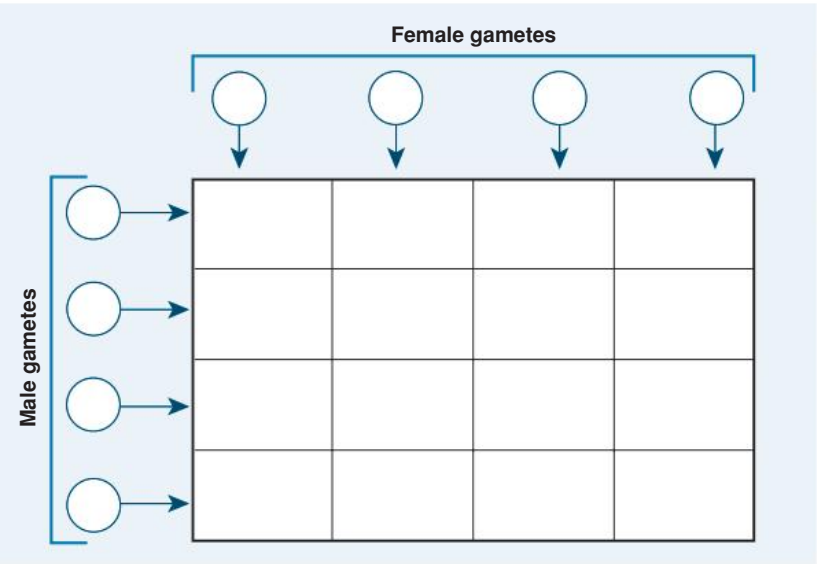
   _____

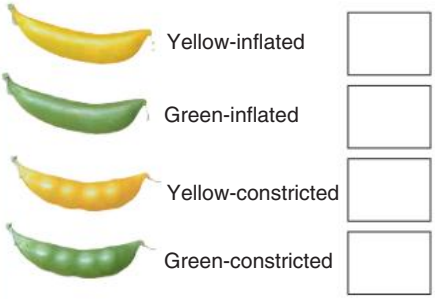


2. The following dihybrid cross shows the inheritance of colour and shape in pea pods. Yellow (**Y**) is dominant over green (**y**) and an inflated pod (**F**) is dominant over a constricted pod (**f**) form.
   (a) Describe the appearance (phenotype) of pea pods with the genotype YyFf: _____

   _____

   (b) Complete the Punnett square below when two seeds with the YyFf genotype are crossed. Indicate the number of each phenotype of the pea pods in the boxes on the right.





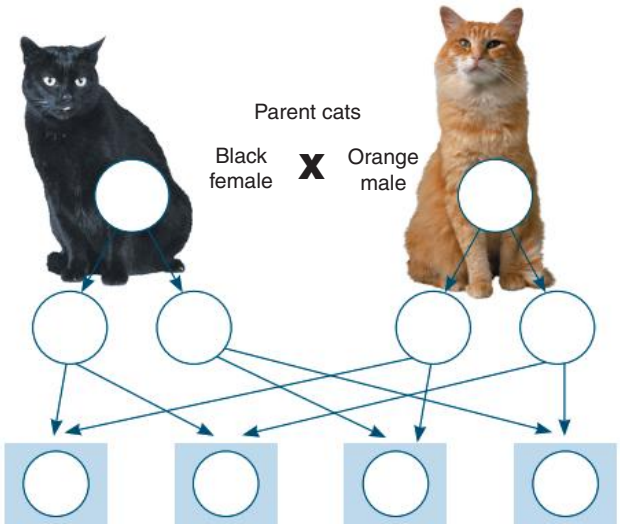
3. One of the gene loci controlling coat colour in cats is sex-linked. The two alleles, red and non-red (or black), are found only on the X-chromosome. Use the alleles listed to answer the following questions:

Allele types		Genotypes		Phenotypes
X_o =	Non-red (=black)	X_oX_o, X_oY	=	Black coated female, male
X_O =	Red	X_OX_O, X_OY	=	Orange coated female, male
		X_OX_o	=	Tortoiseshell, i.e. mix of black and orange in fur (female cats only)

An owner of a cat is thinking of mating her black female cat with an orange male cat. Before she does this, she would like to know what possible coat colours could result from such a cross. Use the symbols above to fill in the diagram on the right. Summarise the possible genotypes and phenotypes of the kittens in the tables below.

	Genotypes	Phenotypes
Male kittens		

Female kittens		



ISBN: 978-1-98-856655-9

CHAPTER 5

Inheritance Patterns in a Population

Key terms

bioinformatics
DNA profiling
DNA sequencing
gel electrophoresis
genetic screening
genome mapping
haplotype
marker gene
microsatellite
multiregional hypothesis
Out-of-Africa hypothesis
polymerase chain reaction
single nucleotide polymorphism (SNP)

Inquiry question: *Can population genetic patterns be predicted with any accuracy?*

Activity number

Genetic technologies

Key skills and knowledge

Image: Bone clones

Population genetics

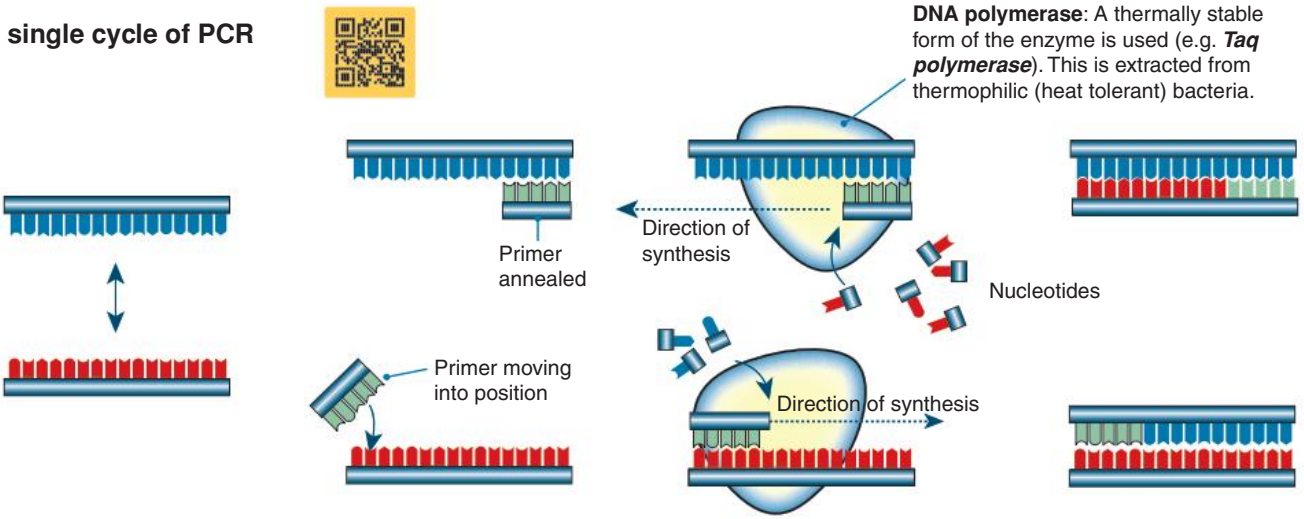
Key skills and knowledge

96 DNA Amplification Using PCR

Key Idea: PCR uses a polymerase enzyme to copy a DNA sample, producing billions of copies in a few hours.

Often, it is very hard to get enough DNA to analyse, e.g. DNA from a crime scene or from an extinct organism. Researchers need to increase the amount of DNA they have to work with. This is done using **polymerase chain reaction** (PCR) which can make billions of copies of a DNA sequence of interest so that it can be analysed. The technique is carried out *in vitro* rather than in a living organism. An overview of PCR given below.

A single cycle of PCR



1. Denaturing
A DNA sample (called target DNA) is obtained. It is denatured (DNA strands are separated) by heating at 98°C for 5 minutes.

2. Annealing
The sample is cooled to 60°C. Primers are annealed (bonded) to each DNA strand. In PCR, the primers are short strands of DNA; they provide the starting sequence for DNA extension.

3. Extension/elongation
Free nucleotides and DNA polymerase are added. DNA polymerase binds to the primers and synthesises complementary strands of DNA, using the free nucleotides.

4. Completed strands
After one cycle, there are now two copies of the original DNA.

Repeat cycle of heating and cooling until enough copies of the target DNA have been produced

1. Outline the process of PCR: _____

2. (a) Explain the purpose of PCR: _____

   (b) Give two examples where PCR is needed to amplify DNA: _____

3. After only two cycles of replication, four copies of the double-stranded DNA exist. Calculate how much a DNA sample will have increased after:

   (a) 10 cycles: _____ (b) 25 cycles: _____

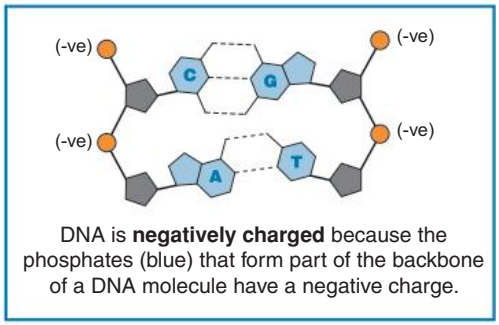
4. Researchers take great care to avoid DNA contamination during PCR preparation. Explain why: _____

ISBN: 978-1-98-856655-9

97 Gel Electrophoresis

Key Idea: Gel electrophoresis is used to separate DNA fragments on the basis of size.

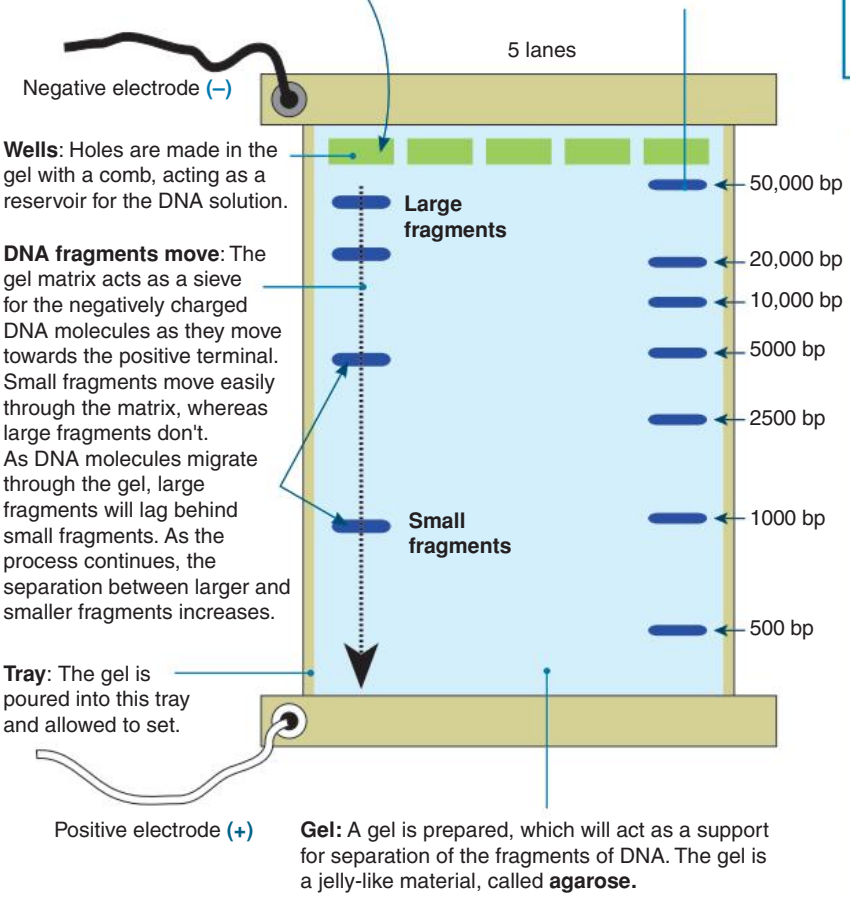
Gel electrophoresis is a tool used to isolate DNA of interest for further study. It is also used for DNA profiling, i.e. comparing individuals based on their unique DNA banding profiles. DNA has an overall negative charge so, when an electrical current is run through a gel, the DNA moves towards the positive electrode. The rate at which the DNA molecules move through the gel depends primarily on their size and the strength of the electric field. The gel they move through is full of pores (holes). Smaller DNA molecules move through the pores more quickly than larger ones. At the end of the process, the DNA molecules can be stained and visualised as a series of bands. Each band contains DNA molecules of a particular size. The bands furthest from the start of the gel contain the smallest DNA fragments.



DNA is **negatively charged** because the phosphates (blue) that form part of the backbone of a DNA molecule have a negative charge.

DNA solutions: Mixtures of different sizes of DNA fragments are loaded in each well in the gel.

DNA markers, a mixture of DNA molecules with known molecular weights (size) are often run in one lane. They are used to estimate the sizes of the DNA fragments in the sample lanes. The figures below are hypothetical markers (bp = base pairs).



Steps in the process of gel electrophoresis of DNA

1. The gel is placed in an electrophoresis chamber and the chamber is filled with buffer, covering the gel. This allows electric current from the electrodes to flow through the gel.
2. DNA samples are mixed with a 'loading dye' to make the DNA sample visible. The dye also contains glycerol or sucrose to make the DNA sample heavy so that it will sink to the bottom of the well.
3. The gel is covered, electrodes are attached to a power supply and turned on.
4. When the dye marker has moved through the gel, the current is turned off and the gel is removed from the tray.
5. DNA molecules are made visible by staining the gel with methylene blue or ethidium bromide which binds to DNA and will fluoresce in UV light.
6. The band or bands of interest are cut from the gel and dissolved in chemicals to release the DNA. This DNA can then be studied in more detail, e.g. its nucleotide sequence can be determined.

1. What is the purpose of gel electrophoresis? _____

_____

_____

2. Describe the two forces that control the speed at which fragments pass through the gel:

   (a) _____

   (b) _____

3. Why do the smallest fragments travel through the gel the fastest? _____

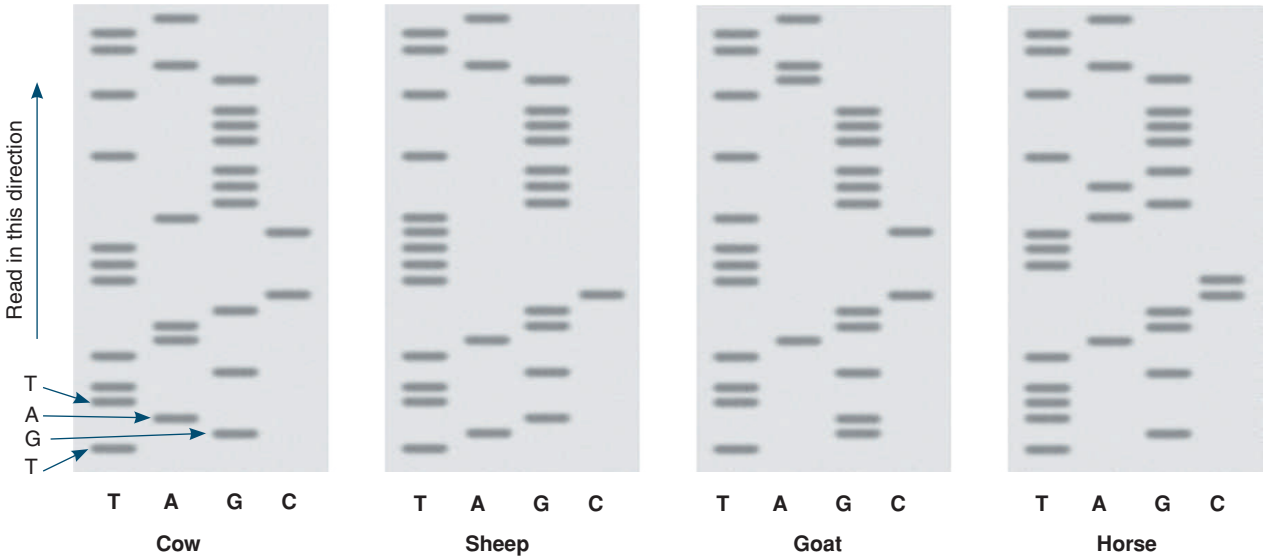
_____

_____

ISBN: 978-1-98-856655-9

Interpreting electrophoresis gels

- Once made, an electrophoresis gel must be interpreted. If a specific DNA base sequence is being investigated, then the band pattern can be used to determine the DNA sequence and the protein that it encodes. Alternatively, depending on how the original DNA was treated, the banding pattern may be used as a profile for a species or individual.
- Commonly, the gene for cytochrome oxidase I (COXI), a mitochondrial protein, is used to distinguish animal species. The genetic information from this gene is both large enough to measure differences between species and small enough to have the differences make sense, i.e. the differences occur in small regions and aren't hugely varied.



4. The photographs above show gel electrophoresis results for four species.

   (a) For each of the species determine the sequence of DNA:

   Cow DNA sequence: _____

   Sheep DNA sequence: _____

   Goat DNA sequence: _____

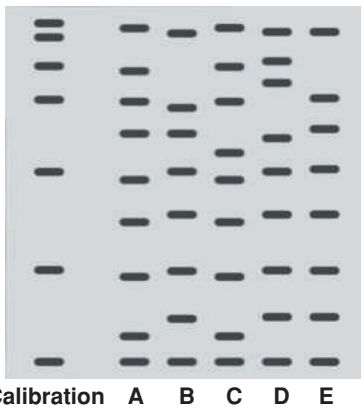
   Horse DNA sequence: _____

   Based on the number of differences in the DNA sequences:

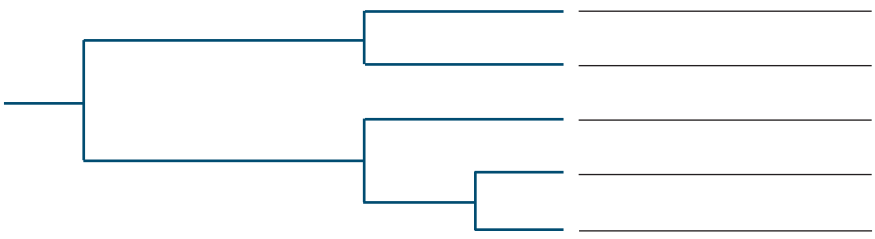
   (b) Identify the two species that are most closely related: _____

   (c) Identify the two species that are the least closely related: _____

5. What makes COXI useful for comparing species by gel electrophoresis? _____



6. Determine the relatedness of each individual (A-E) using each banding pattern on the set of DNA profiles (left). When you have done this, complete the phylogenetic tree by adding the letter of each individual.

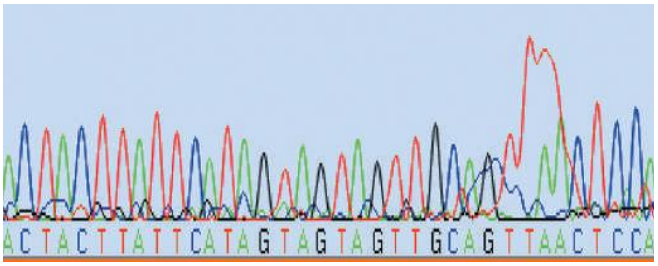


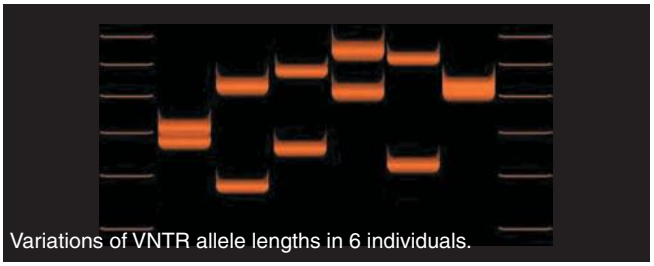
ISBN: 978-1-98-856655-9

98 Applications of DNA Analysis

Key Idea: DNA profiling involves the comparison of DNA samples. DNA sequencing is the process of determining the order of nucleotides in DNA.

DNA can be analysed in different ways depending on what information a researcher is seeking. **DNA sequencing** determines the sequence of nucleotides (A, T, C, and G) in a gene or section of DNA. Sequencing has many applications in science, including molecular biology, evolutionary biology, and medicine. **DNA profiling** looks at a specific DNA pattern (profile) and compares it to another.




Variations of VNTR allele lengths in 6 individuals.

DNA sequencing

DNA sequencing can be used to sequence all of an organism's genetic material (its genome). Large genomes must, initially, be broken down into small pieces and amplified using PCR. In chain termination methods (Sanger technology), the terminator end of the DNA fragments have fluorescent labels attached and the fragments are separated using gel electrophoresis. Each type of nucleotide base fluoresces a different colour, so when the gel is scanned the DNA sequence can be obtained (above). Current high-throughput sequencing technologies have automated this basic process, running systems in parallel and producing thousands or millions of sequences at a time.

Once the sequence is obtained, the information can be used to:

- Locate specific genes, if associated with a disease, and target genes in gene therapy, i.e. correcting a defective gene.
- Determine the function of a gene.
- Provide information on the evolutionary history and/or relatedness between species.
- Improve genetic modification techniques.

DNA profiling

DNA profiling is also called DNA fingerprinting. Unlike sequencing, profiling does not attempt to determine the DNA sequence. Instead, the DNA profiles of individuals are compared to determine if a sample of DNA has come from a particular individual or not.

Within DNA there are regions containing short repeating DNA sequences. The sequences, called short tandem repeats (STRs), are only 2-6 base pairs long. STRs can repeat up to 100 times so there is STR variation between individuals. This variation can be used to identify a person's unique STR profile. Longer sequences of tandem repeats, called variable number tandem repeats (VNTRs), are also used in profiling (above).

Two common applications of DNA profiling are:

- Paternity testing (determining who the father of a child is).
- Forensic investigations. The DNA profile of a suspect is compared to DNA evidence collected at a crime scene to see if they were involved in the crime.

1. Explain the difference between DNA sequencing and DNA profiling: _____

2. There are about 3.2 billion base pairs in the human genome. 99.9% of the genome is identical between humans. Calculate the number of base pairs that are variable in humans:

3. (a) What are short tandem repeats? _____

   (b) Explain how STRs cause variation between individuals: _____

ISBN: 978-1-98-856655-9

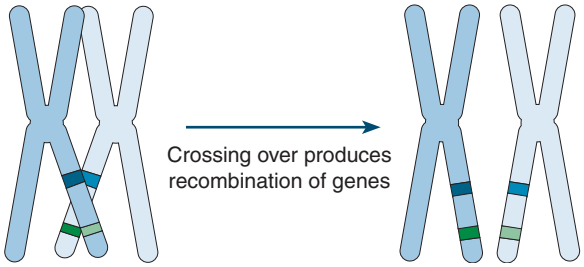
99 Genome Mapping

Key Idea: A genome map identifies key markers in an organism's genome and ultimately identifies the positions of genes on chromosomes.

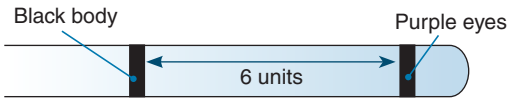
Gene mapping has provided information on the locations of genes on a chromosome and has been an important tool in the identification of genetically linked diseases. There are two general methods of genome mapping. Genetic mapping looks at recombination frequency during meiosis. Physical mapping uses overlapping sequenced sections of DNA to build a physical map of the chromosome.

Genetic mapping

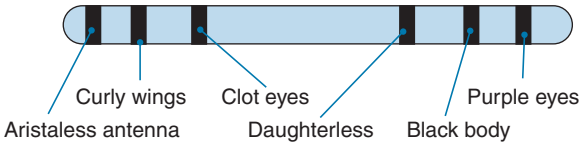
- Recall that during meiosis non-sister chromatids of homologous chromosomes exchange information (crossing over). This can be detected in the offspring as recombination. Using the frequency at which recombinants appear in the offspring it is possible to work out the positions of various genes. This method is notable in that it was used to map the chromosomes of the fruit fly, *Drosophila*.



- Genetic mapping can be illustrated using the traits for black body and purple eyes in *Drosophila*. Both are caused by recessive alleles. A heterozygous fly is mated with a homozygous recessive fly and the phenotypes of the offspring are observed.
- If, out of 281 offspring, the following phenotypes were observed: 139 normal body and eyes (same as the heterozygous parent), 125 black body and purple eyes (same as the homozygous recessive parent), 8 normal body and purple eyes, and 9 black body and normal eyes, then there was a total of 17 recombinant offspring.
- Calculating the crossover value gives the distance between the genes in map units: (17 ÷ 281) x 100 = 6.

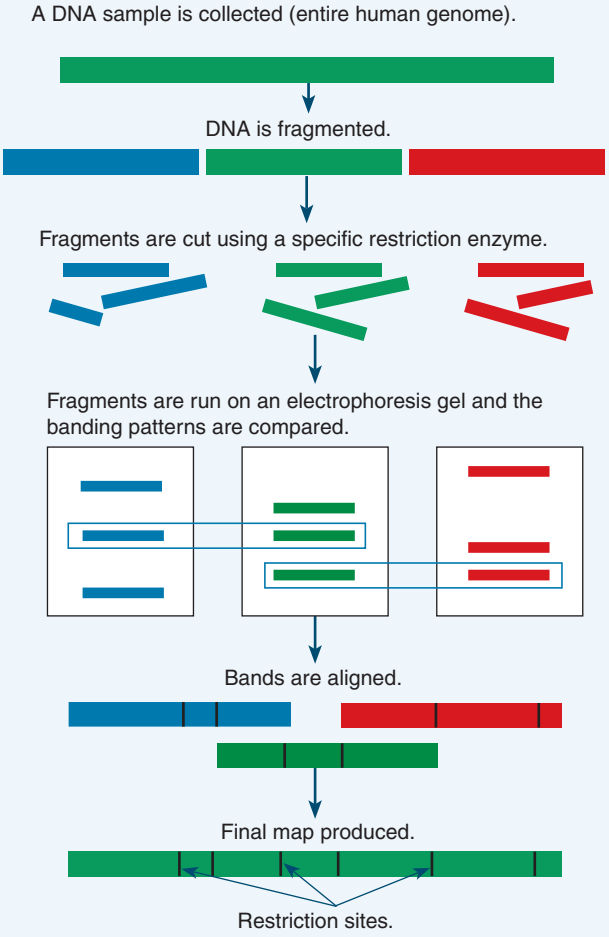


- By doing this with many genes it is possible to work out where genes are on the chromosomes.



Physical mapping

There are many methods used for physical mapping. They include restriction mapping, fluorescent in situ hybridisation (FISH) mapping, and sequence tagged site (STS) mapping. Each mapping type shows something specific about the genome. For example, restriction mapping cuts the DNA using a specific restriction enzyme which cuts the DNA at a specific sequence (e.g. GAATTC).



1. What is the purpose of genome mapping? _____

_____

_____

2. (a) What is the difference between genetic mapping and physical mapping? _____

_____

_____

(b) Identify an advantage and disadvantage of each method: _____

_____

_____

ISBN: 978-1-98-856655-9

100 Bioinformatics

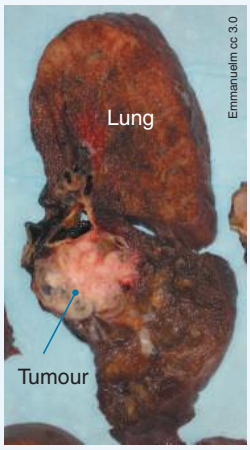
Key Idea: Bioinformatics uses powerful computing and mathematical tools to collect, store, and analyse large amounts of biological information.

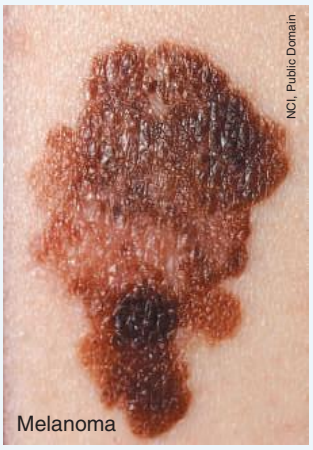
Bioinformatics is the collection, storage, and mathematical analysis of biological information using computers. It is often used in the fields of molecular phylogenetics and genome studies. The information is stored in databases where it can easily be retrieved, analysed, and compared. Improved technology (e.g. faster computing power) allows large amounts of data to be analysed very quickly. Bioinformatics has been important in analysing data from the Human Genome Project. Some bioinformatics applications are described below.

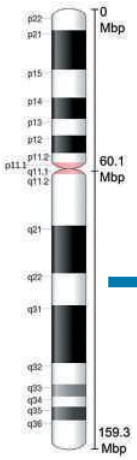
Using bioinformatics and genome analysis to tackle cancer

The Cancer Genome Atlas (**TCGA**) is a project which aims to improve the diagnosis, treatment, and prevention of cancer. During the 12 years the project has been running it has:

- Collected data from 11,000 patients.
- Produced over 2.5 petabytes of data.
- Characterised 33 different types of cancers.
- Used the data to detect cancerous mutations, identify cancer markers, and understand the molecular and functional basis of tumour behaviour.
- Provided information for the development and delivery of highly specific cancer drugs (see below).

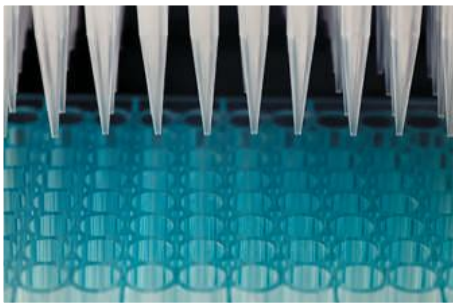






The EGFR gene is located between 55 and 55.2 Mbp on the p arm of chromosome 7.

Sequencing and gene mapping (knowing a gene's location) allows researchers to identify mutations that can cause cells to become cancerous. For example, mutations causing over-expression of the EGFR gene causes many cancers.



Understanding the role of specific genes in cancer helps researchers develop therapeutic drugs. The use of bioinformatics and high throughput screening of potential new drugs means many drugs can be developed and tested for effectiveness in a short space of time. Researchers use the information from the HGP to develop drugs to target specific types of cancer. This is called targeted drug therapy.

An ideal targeted drug therapy acts on a specific type of abnormal cell while leaving normal cells unaffected. Often these drugs have fewer side effects as a result. Iressa® (above) is an example of targeted drug therapy. Iressa is designed to treat cancerous cells in the lung with a mutation to the EGFR gene. Iressa® blocks signals to the cancer cell telling it to grow and divide. It will not work on tumours without the EGFR mutation.

1. What is bioinformatics? _____

2. How has bioinformatics made projects like The Cancer Genome Atlas possible? _____

3. Using an example, explain how detailed genomic information can be used for targeted therapy: _____

ISBN: 978-1-98-856655-9

101 Genome Sequencing For All

Key Idea: Many companies offer cheap genetic testing directly to the public. However, the testing procedure and interpretation are not always accurate.

Several companies sell cheap DNA test kits directly to the public, often for less than $300. The purchaser simply sends a saliva sample off for analysis. The most common tests purchased provide information about ancestry, but some also test for genetic health risks. The tests are very popular, and consumers often see them as harmless fun. But people often don't think about some of the serious consequences of them. How reliable are the results and what happens to your information once it is collected?

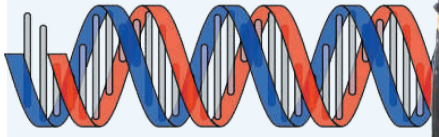
What does my DNA tell me?

People use DNA testing to find out all sorts of information about themselves. This includes:

- Where did my ancestors come from?
- What diseases could I develop in the future?
- Could I pass on an inherited disease to my future children?
- Who is my father?

Hundreds of companies, including Australian ones, offer to provide answers to these questions. The tests are incredibility popular and millions of people have taken DNA tests, including at least 20,000 Australians.

At the moment, most kits look for specific genes but whole genome sequencing for less than $1000 may be available soon.



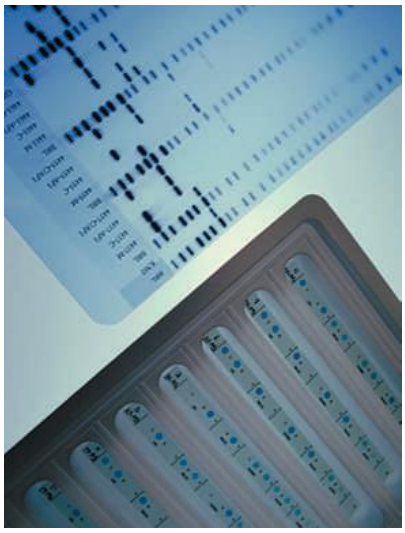
Can I trust the results?

No, not always. Some companies have very rigorous testing procedures while others do not. Recently, a customer sent a sample of his dog's DNA to a laboratory and they didn't notice it wasn't human DNA! There are many examples when people have had their ancestry analysed by different companies and received very different results. Often, this is caused by differences in their reference databases. Different results are returned based on the size and population sample your DNA is compared to.

What if testing shows you have a gene linked to a certain disease? Having a particular gene means there is a risk you could develop the disease, but it does not mean you definitely will. Other factors, such as lifestyle and environment, also contribute. A positive outcome of the discovery is that you could make lifestyle changes and have regular checks to reduce your disease risk and improve your chances of detecting disease. However, a person could spend their lifetime worrying unnecessarily about a disease that may not develop. What if there is no cure for the disease, e.g. Huntington's. What impact would that have on a person's wellbeing and outlook on life?

What happens to your information once it is collected?

Most people assume their genetic information cannot be accessed again or is destroyed, but this is not always the case. 23andMe is one of the world's most popular DNA testing companies. In 2018, they made a very low key announcement that they were selling customers' DNA data to GlaxoSmithKline, a large pharmaceutical company. Consumers had to scroll through pages of terms and conditions to find this information in the small print. 23andMe states that the data was being on-sold to the pharmaceutical company so that new drugs could be developed. More recently, 23andMe has added an opt out option for its customers but many other companies haven't. What does this mean for you?



- The testing company is making money by selling customer data but the customer gets no money.
- Your personal information is being shared with other parties. Your unique genetic information could be shared and used anywhere without your consent.
- Several studies have shown it is actually possible to track an individual person within a large database, based on their genetic information. This information could be misused. For example, a political candidate may be disadvantaged because they have a risk of developing Alzheimer's.
- In Australia, life insurance companies can ask if you have had a DNA test, or if you are considering having a test. They can refuse to give you cover if there are any concerning health markers present. This is called genetic discrimination.

1. Work in pairs to discuss the pros and cons of cheap genetic testing, then join with another pair to hear their discussion points. At the end of your discussion decide whether you think DNA testing kits are a good or bad idea.

   Summarise your ideas and staple them to this page.

ISBN: 978-1-98-856655-9

102 Applications of DNA Profiling

Key Idea: DNA profiling has many forensic applications, from identifying criminal offenders to saving endangered species.

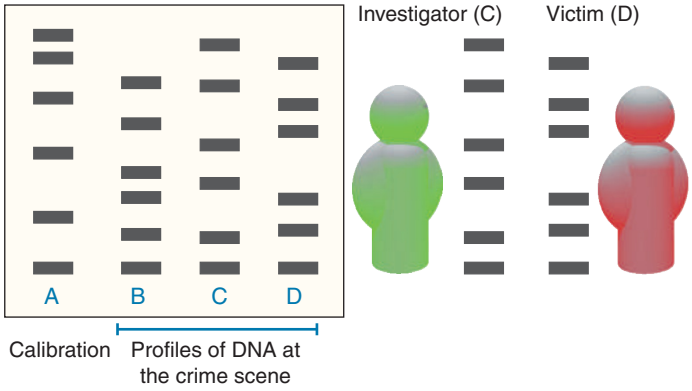
The use of DNA as a tool for solving crimes such as homicide is well known, but it also has several other applications. DNA evidence has been used to identify body parts; solve cases of industrial sabotage and contamination; test paternity; and even identify animal products made, illegally, from endangered species.

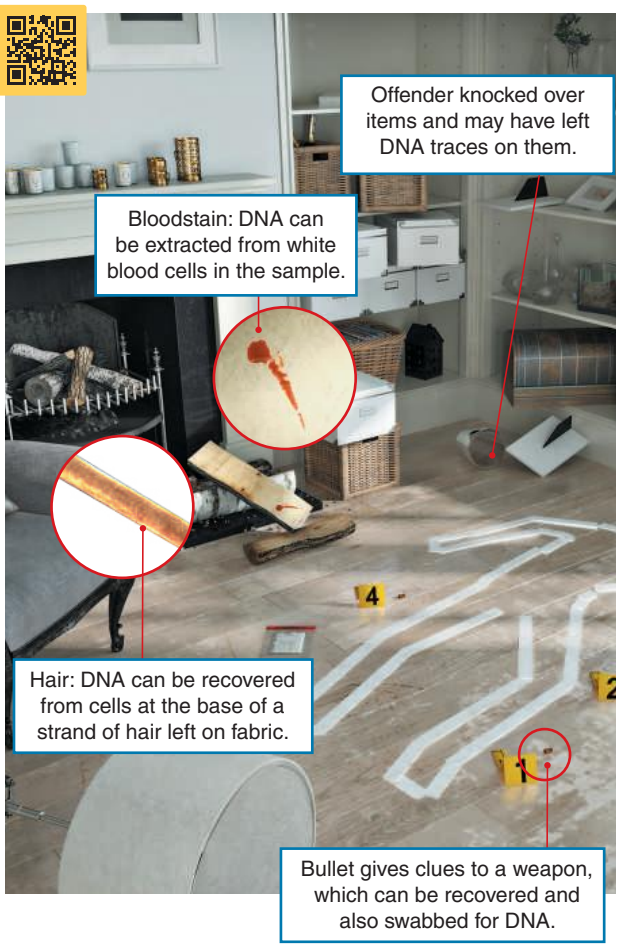
Using DNA to solve crimes

Although it does not make a complete case on it own, DNA profiling, in conjunction with other evidence, is one of the most powerful tools in identifying offenders or unknown tissues.

A lot of DNA is found at crime scenes and the information collected can be used to help identify the criminal. However, not all of the DNA collected will be from the criminal. Other DNA could belong to the victim, to people who came to their aid, e.g. paramedics, or to the police investigators, if they have not taken correct precautions.

In the example (right) the criminal who broke into this home has left behind several samples of their DNA. Samples of material that may contain DNA are taken for analysis. At a crime scene, this may include blood and body fluids as well as samples of clothing or objects that the offender might have touched. Samples from the victim and the investigator are also taken to eliminate them as a possible source of contamination (below). In this example, the DNA of the people who live in the house will also be collected so their profiles can be eliminated. A calibration or standard is run so the technician knows the profile has run correctly.

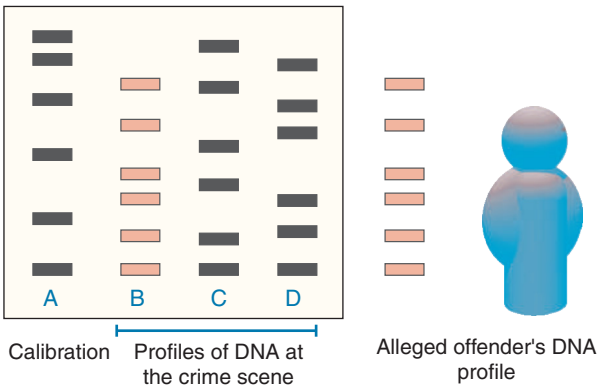




There are two different ways an offender can be identified through DNA profiling.

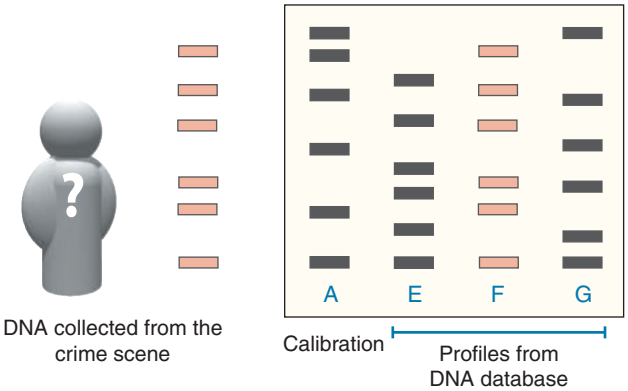
1. If a person is suspected of a crime, a sample of their DNA can be taken, e.g. blood sample, and compared to DNA evidence collected at the crime scene. A match indicates they are the offender. If there is no match, the person can be cleared as a suspect.
2. In cases where the suspect is unknown, biological evidence from the crime scene is analysed and the profile is compared to known offender profiles in DNA databases. The profile may match that of a known offender.

1 A person is suspected of the crime



Match! The alleged offender's profile matches the DNA collected at the crime scene.

2 The offender is unknown



Match! The DNA collected from the crime scene matches the profile of a known offender in the database.

ISBN: 978-1-98-856655-9

Paternity testing

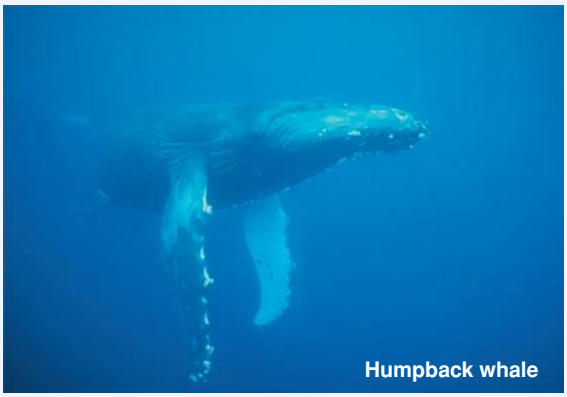
DNA profiling can be used to determine paternity (and maternity) by looking for matches in alleles between parents and children. It is used in cases such as child support or inheritance. DNA profiling can establish the certainty of paternity (and maternity) to a 99.99% probability of parentage.

Every short tandem repeat (STR) allele is given the number of repeats as its name, e.g. 8 or 9. In a paternity case, the mother may be 11, 12 and the father may be 8, 13 for a particular STR. The child will have a combination of these. The table below illustrates this:

DNA marker	Mother's alleles	Child's alleles	Father's alleles
CSF1PO	7, 8	8, 9	9, 12
D10S1248	14, 15	11, 14	10, 11
D12S391	16, 17	17, 17	17, 18
D13S317	10, 11	9, 10	8, 9

The frequency of the each allele occurring in the population is important when determining paternity (or maternity). For example, DNA marker CSF1PO allele 9 has a frequency of 0.0294 making the match between father and child very significant, whereas allele 12 has a frequency of 0.3446, making a match less significant. For each allele, a paternity index (PI) is calculated. These indicate the significance of the match. The PIs are combined to produce a probability of parentage. 10-13 different STRs are used to identify paternity. Mismatches of two STRs between the male and child is enough to exclude the male as the biological father.

Whale DNA: tracking illegal slaughter



Humpback whale

Under International Whaling Commission regulations, some species of whales can be captured for scientific research and their meat can be sold legally. Most whales, including humpback and blue whales, are fully protected and to capture or kill them is illegal.

Between 1999 and 2003, researchers used DNA profiling to investigate whale meat sold in markets in Japan and South Korea. They found 10% of the samples tested were from fully protected whales, including western grey whales and humpbacks. They also found that many more whales were being killed than were being officially reported.

1. Why are DNA profiles obtained for both the victim and investigator? _____

_____

_____

2. Study the profile on the right.

   (a) Is the alleged offender innocent or guilty?_____

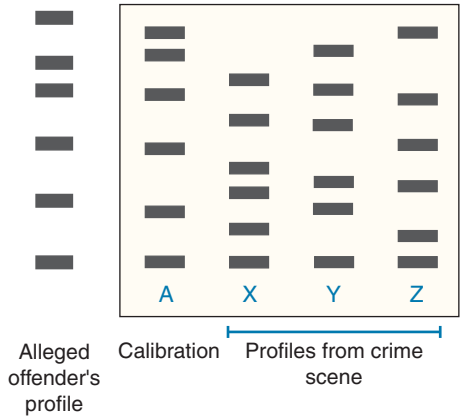
   (b) Explain your decision: _____

_____

_____

_____

_____



3. For the STR D10S1248 in the example above, what possible allele combinations could the child have?

_____

4 A paternity test was carried out and the abbreviated results are shown (right):

   (a) Could the man be the biological father? _____

   (b) Explain your answer: _____

_____

_____

_____

DNA marker	Mother's alleles	Child's alleles	Man's alleles
CSF1PO	7, 8	8, 9	9, 12
D10S1248	14, 15	11, 14	10, 11
D19S433	9, 10	10,15	14, 16
D13S317	10, 11	9, 10	8, 9
D2S441	7, 15	7, 9	14, 17

ISBN: 978-1-98-856655-9

103 How Well Does DNA Profiling Work?

Key Idea: The type of DNA sample influences the success of DNA profiling in forensic cases.

DNA profiling to identify an offender at a crime scene is an important aspect of forensic investigation. To cope with the large number of samples analysed in Australia, investigators are continually improving their processes. They need to increase throughput but still maintain the accuracy and reliability of data produced.

Efficiency cannot be increased at the expense of reliability. Identifying the wrong person could mean they are convicted for a crime they didn't commit, while the person who committed the crime goes unpunished, and may offend again. Several factors increase the chances of obtaining a successful profile. One factor is the type of sample used to obtain the DNA (below).

How does sample type affect profiling success?

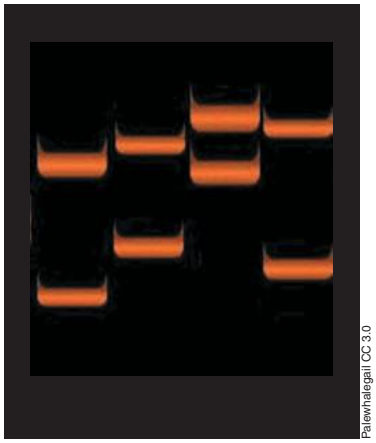
Between 2012 and 2013, new DNA analysis kits were introduced into forensic laboratories in Australia. The new kits analysed more DNA markers than the old kits. A project was carried out to compare the results from the old kits with those from the new kits.

One of the study areas examined how sample type affected the success rate. These results are shown in Table 1. The criterion for success was a match of more than 6 alleles. When fewer than 6 allele matches were obtained, the match was recorded as unsuccessful. DNA analysis was carried out using blood, saliva, and trace DNA samples. Trace DNA is any sample that falls below the recommended thresholds for the analysis, and cannot be defined by a precise picogram amount.

Table 1. DNA profile success rates from different samples using two different types of DNA analysis kits, the older Profiler Plus and newer Powerplex 21.

	Sample type	Profiler plus kit		Powerplex 21 kit	
		Number of items	Successful profiles (%)	Number of items	Successful profiles (%)
Swab	Blood	43	92.6	20	90.4
Clothing	Blood	3	100.0	6	100.0
Cigarette butt	Saliva	53	71.1	9	66.6
Mouth/rim bottle	Saliva	54	68.5	1	100.0
Clothing	Trace	50	70.0	57	50.8
Items: Probable friction	Trace	16	37.5	41	48.7
Items: No probable friction	Trace	27	55.5	133	46.6

Data: DNA Profiling success rates on volume crime cases to determine the optimal number and type of samples that should be analysed per case.
Linzi Wilson-Wilde, Mojca Kegolvic, and Simon Walsh. Presented to the 25th Congress of the International Society for Forensic Genetics, 2 – 7 September 2013.



Palewhalegail CC 3.0

1. Explain why it is important that a fast analysis time is balanced with high accuracy rate: _____

2. Table 1 shows the successful rates of DNA profiles obtained from a number of different sample types.

   (a) Identify the sample type with the highest successful profiles: _____

   (b) Identify which sample type is the least likely to produce a successful profile: _____

   (c) Explain why the sample you named in (b) is the less likely to produce a successful profile: _____

ISBN: 978-1-98-856655-9

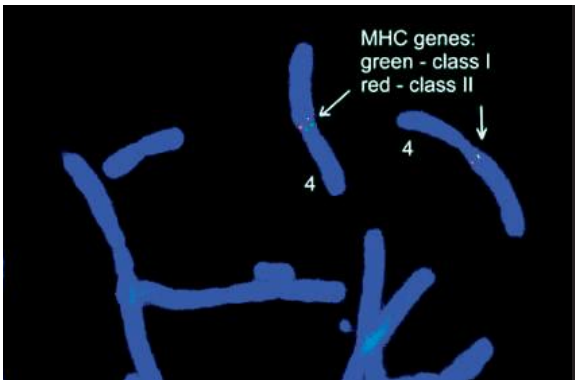
104 Population Genetics in Conservation

Key Idea: Knowing the genetic diversity of a population can help conservation breeding programmes.

The genetic diversity of Tasmanian devils is particularly low and it faces numerous conservation concerns, including devil facial tumour disease. This case study explores the relationship between MHC allelic diversity and how it affects reproductive success in Tasmanian devils. The MHC genes control self-recognition in the immune system.

Mike Lehmann cc 3.0

Tasmanian devils are the largest surviving marsupial carnivore. Although now restricted to Tasmania, devils were once found throughout mainland Australia but became locally extinct about 3000 years ago. Genetic evidence suggests that the devils went through at least two historic population crashes, one about 30,000 years ago and another about 3000 years ago. Coupled with these historic declines are modern declines (1850 to 1950) as a result of trapping and disease.



Cheng et al. For full credit, see photocredits

MHC genes are located on chromosome 4 of the Tasmanian devil genome.

1. **PREAMBLE**

   The data on the right is part of a study on the breeding success of a group of captive Tasmanian devils. The data shows the age of the male and female in the pair, the reproductive success of the pair, and the number (out of 6) of MHC class-I heterozygous loci (location of microsatellite alleles) of each male and female in the pair.

   You will analyse this data to determine the effect of two separate variables on the breeding success of Tasmanian devils and use your analysis to recommend the best course of action to increase reproductive success in devils.

   The variables you will investigate are female age and genetic diversity.

   You may require extra paper to work through and organise the data into meaningful groups. Attach all extra paper to this page. Alternatively, the data could be entered into a spreadsheet.

Female		Male		Reproductive success	A
No. hetero-zygous loci	Age (Yr)	No. hetero-zygous loci	Age (Yr)		
4	4	1	4	successful	
4	3	2	3	unsuccessful	
4	3	3	3	unsuccessful	
3	2	2	3	unsuccessful	
4	3	1	5	successful	
4	2	3	3	successful	
6	4	1	5	successful	
6	2	3	3	successful	
3	2	4	2	unsuccessful	
3	2	3	3	unsuccessful	
4	3	2	2	unsuccessful	
1	3	4	3	successful	
4	3	3	2	unsuccessful	
4	3	3	4	unsuccessful	
4	2	1	2	successful	
6	2	2	2	successful	
6	4	3	4	successful	
5	2	1	3	successful	
5	3	2	3	successful	
5	3	2	3	successful	
3	2	4	4	successful	
1	2	4	3	successful	
1	3	3	3	successful	
5	2	2	3	successful	
5	2	1	3	successful	
5	4	1	3	unsuccessful	
5	4	2	3	unsuccessful	
2	2	3	2	successful	
2	4	2	4	unsuccessful	
5	2	0	2	successful	
3	2	4	2	successful	
3	5	2	5	unsuccessful	
1	3	1	5	unsuccessful	
1	2	4	2	successful	
1	3	3	3	unsuccessful	
2	2	4	2	successful	
5	3	4	4	unsuccessful	
5	2	1	2	unsuccessful	
1	2	4	3	successful	
2	3	4	4	unsuccessful	
2	4	2	4	unsuccessful	
3	2	4	3	successful	
5	3	2	2	successful	
5	5	2	5	unsuccessful	
3	3	6	4	unsuccessful	
2	2	2	3	successful	
2	3	2	3	unsuccessful	
5	2	4	3	successful	
5	3	3	4	successful	

MHC diversity and female age underpin reproductive success in an Australian icon; the Tasmanian devil
Tracey Russell *et al* www.nature.com, 8 March 2018 CC 4.0

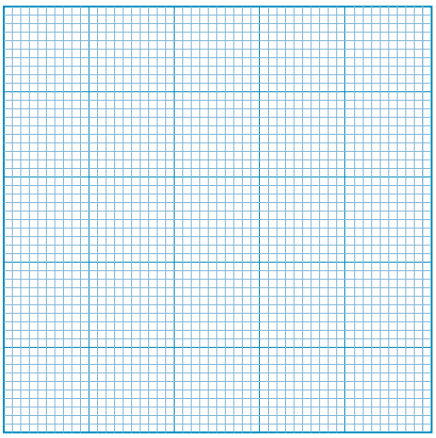
ISBN: 978-1-98-856655-9

2. **Effect of female age**

(a) To analyse the effect of female age you need to organise the data into female age groups (from 2 years to 5 years), then into successful or unsuccessful breeding. A tally chart may be useful:

Age group (female)	2	3	4	5
Successful				
Unsuccessful				
Total				
% Successful				

(b) Produce a scatter plot of the result on the grid below (age on the x axis, % success on the y axis).



3. **Effect of heterozygous loci**

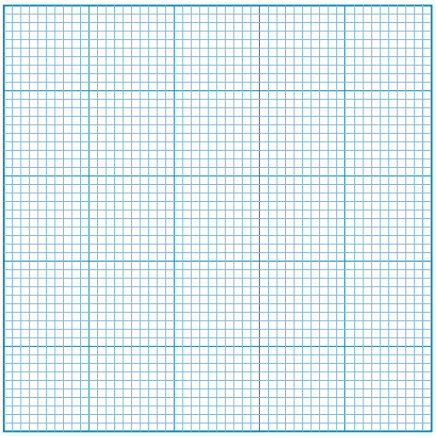
There is a body of evidence to suggest that greater heterozygosity correlates with greater breeding success. Here you will analyse the effect of the absolute difference in heterozygosity of the 6 MHC loci (male and female). For example the male may be heterozygous for 2 loci and the female may be heterozygous for 4 loci. The absolute difference in heterozygosity is therefore 2.

(a) For each pairing, calculate the absolute difference in heterozygous loci. Write this in column A in the table on the previous page.

(b) Now use a tally chart, as before, to group successful and unsuccessful pairings according to the absolute difference in heterozygosity:

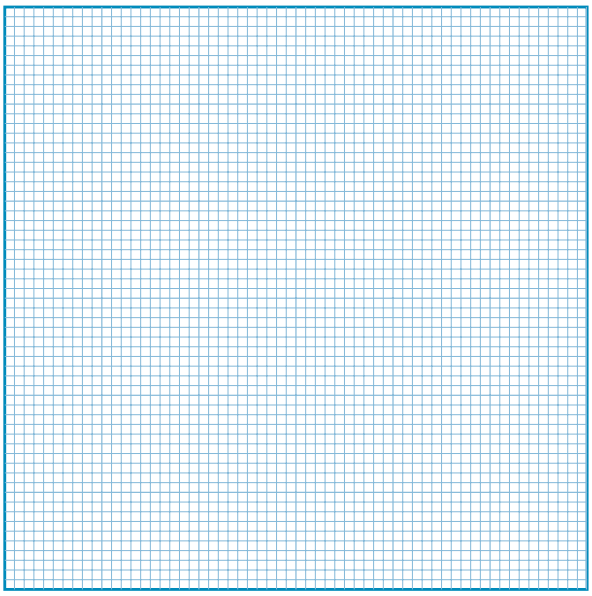
Absolute difference in heterozygosity	0	1	2	3	4	5
Successful						
Unsuccessful						
Total						
% Successful						

(c) Produce a scatter plot for the result on the grid below (absolute difference on the x axis, % success on the y axis).



ISBN: 978-1-98-856655-9

4. **Comparison of male and female heterozygosity**
   For the successful breeding pairs, plot a graph of the number of female heterozygous loci (x axis) vs the number of male heterozygous loci (y axis)



5. Finally, calculate the mean age of females that bred successfully and unsuccessfully, and the average absolute difference in heterozygosity for successful and unsuccessful pairings:

6. Describe the relationship between female age and breeding success:

7. Describe the relationship between the difference in heterozygosity and breeding success:

8. Describe the relationship between the number of heterozygous loci of the male and female in a successful breeding pair:

9. (a) Based on your analysis, what would you recommend to increase the breeding success of captive Tasmanian devils?

   (b) Suggest other analysis of the data that may provide insight into breeding success:

ISBN: 978-1-98-856655-9

105 Population Genetics and Disease Inheritance

Key Idea: Genetic studies can give insight into the effect of genes and the environment in the onset of disease.

Gathering and analysing genetic data helps scientists develop an understanding of the influence of genes on certain diseases. Some diseases have a direct gene cause e.g. from a single gene mutation, or they may be associated with the interactions of genes and the environment. Gene screening uses multiple methods for identifying genetically linked diseases in people. Genetic testing provides specific DNA analysis of a targeted gene. The data gathered may be used in population wide studies to help understand the prevalence of particular gene mutations and the diseases they are associated with, as well as lifestyle choices that increase the risk of triggering disease such as cancer.

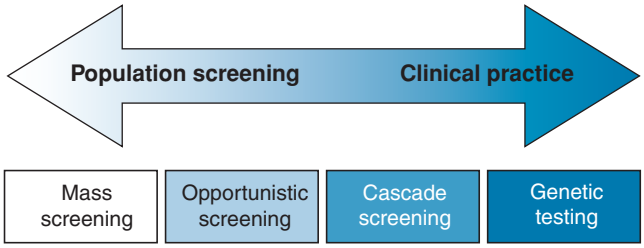
Genetic screening programmes

These are usually population wide programmes and are used as early diagnosis tests, often soon after birth. They include the following tests:

- Tests for current conditions with a known genetic cause.
- Predictive screening, i.e. screening for genes that may cause diseases in an individual's future.
- Carrier screening: testing to find if an asymptomatic person carries a recessive disorder.

Some of these programs are mandatory (e.g. PKU, hypothyroidism, and cystic fibrosis) and are performed within 48 hours of birth. Others are voluntary programmes, for example, for people who may carry a genetic disorder and want to establish the risks of passing it to their offspring. Sometimes the genetic screening programmes are undertaken to provide data about the frequency of a genetic disorder in the wider population, e.g. cystic fibrosis has a carrier frequency of about 1 in 25 people.

Genetic screening continuum



Genetic screening tests can be placed on a continuum. The wider ranging, less detailed tests are used for mass screening of the population. More specific tests are done in specialist clinics and when needed. Opportunistic testing is performed when individuals enter the health system for an unrelated illness and tests show possible other problems. Cascade screening is the systematic testing of individuals related to a person identified as a carrier or sufferer of a genetic disorder.

An example of a genetic screening programme

Phenylketonuria is a metabolic disorder with serious health and mental consequences if it is not treated. People with PKU can not metabolise phenylalanine, a common amino acid. The disorder is not curable but can be managed by controlling phenylalanine intake in the diet. There may be no symptoms in people who are diagnosed early enough.

Blood obtained from pricking a newborn's heel (heel prick test) and collected on filter paper is used for the PKU test. The most common test for PKU is the Guthrie test. A small disk of the dried blood is placed on an agar plate containing a substance that inhibits bacteria growth. If the blood contains elevated levels of phenylalanine the bacteria begin to grow, allowing PKU to be detected.

USAF

The heel prick collects blood for PKU and other genetic disorders of amino acid metabolism, congenital hypothyroidism, cystic fibrosis, galactosaemia, and fatty acid oxidation defects.

1. Contrast genetic screening and genetic testing: _____

2. Why is a simple test used for screening, while a more detailed test is used for genetic testing?

3. Explain why each of the following would be used:

   (a) Opportunistic screening: _____

   (b) Cascade screening: _____

ISBN: 978-1-98-856655-9

Using genetic studies to identify disease risk and treatment

- Recall that specific groups of single nucleotide polymorphisms (SNPs) found close together are called haplotypes. Population wide studies of haplotypes allow scientists to associate different haplotypes, or groups of haplotypes, with diseases.

Identifying a relationship

- To study the genetic component of a disease, researchers require two main study groups: people with the disease and people without the disease (known as phenotype first).
- A genome wide association study (GWAS) is carried out on each individual in each group. The study looks for single nucleotide polymorphisms or **SNPs** (changes to single base pairs) to see if there are differences between the control group and the afflicted group. There are over 100 million SNPs spread throughout the human genome and there are numerous ways to identify them, including DNA sequencing and using restriction enzymes.
- Once the SNPs are identified, their frequencies are analysed to see if any are significantly different between the groups. SNPs found to be associated with a disease do not always just appear in people with the disease. Often, they can be found in people without the disease. This lack of a definitive link makes linking a disease to a specific DNA profile a matter of probability and risks.

Using profiling to analyse disease risk

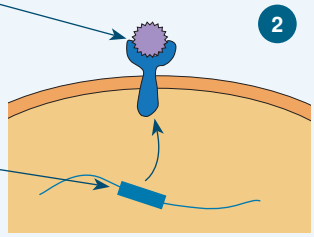
Using DNA profiling as a tool to analyse disease risk is a relatively new field. Although it promises a new way of diagnosing disease, to date it has produced inconsistent results because of the complexity of diseases involving large numbers of genes. One promising use of DNA profiling in disease analysis is the ability to determine response to albuterol in the treatment of asthma.

Albuterol is used to relieve the symptoms of asthma. It works well on some people but not on others.

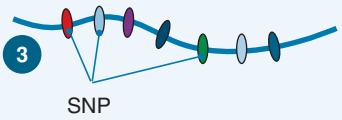
1

Albuterol binds to the beta2 adrenergic receptor in a cell's plasma membrane and causes the relaxation of smooth muscle in the airways.

The beta2 AR protein is encoded by the ADRB2 gene. Scientists wondered if genetic differences in or near this gene affected how well albuterol worked.

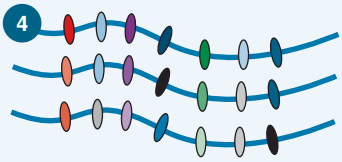


2



3 SNP

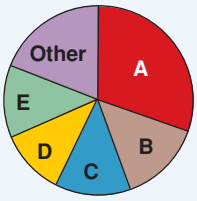
Scientists analysed a 3000 base pair piece of DNA near the ADRB2 gene and identified 13 SNPs.



4

The SNPs are found arranged in combinations called haplotypes. 12 different haplotypes have been identified.

% haplotypes in population



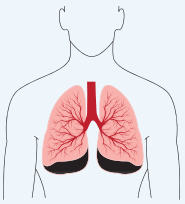
5

Every person has a haplotype profile relating to the ADRB2 gene. Five of the haplotypes are relatively common.

A	Poor
B	Good
C	Fair
D	None
E	Very good

6

Some of the haplotypes seem to be related to the effect of albuterol on people. Some haplotypes are found mainly in people who have a poor response to albuterol (D), while others are found in people who respond well to the drug (B, E).



7

Patient responsive to albuterol.

This data could possibly be used by doctors when prescribing albuterol. In the future, other profiles could be used to determine the effect of other drugs on people.

4. Explain the use of population wide studies of haplotypes: _____

_____

_____

_____

5. (a) Why is a set of SNPs (a haplotype) used when identifying a genetic disease, rather than just a single SNP?

_____

_____

(b) Why is the use of SNPs not a definitive way to assess the risk of a genetic disease? _____

_____

_____

ISBN: 978-1-98-856655-9

Using population studies to identify cancer risk

- Cancer is a leading cause of death. Around 10 million people worldwide die from cancer each year. Although most cancers are related to the environment and lifestyle, e.g. smoking, sunburn etc., around 5% of cancers are related to heritable mutations in certain genes.
- Genes with mutations responsible for heritable cancers include the BRCA1 and BRCA2 genes, commonly related to breast cancer. There are numerous others associated with a range of other cancers.
- It is important to note that although a person may have the mutant allele of the genes associated with cancer, it does not mean they will develop that cancer. Nor does it mean that people who do not have the mutant alleles will not develop cancer. Lifestyle choices play an important part in the development of cancer.
- The table below shows the results of a study that investigated the association of various genes with the incidence of cancer in 64,791 women.

Breast cancer predisposition gene	Cancer patients with cancer allele N=32,247	Percentage of cancer allele in cancer patients	Controls (no cancer) with cancer allele N=32,544	Percentage of cancer allele in controls
ATM	253	*0.78 %*	134	*0.41 %*
BARD1	49		35	
BRCA1	275		37	
BRCA2	417		78	
CDH1	17		6	
CHEK2	349		138	
NF1	19		11	
PALB2	148		38	
PTEN	8		3	
RAD51C	41		35	
RAD51D	26		14	
TP53	19		2	
Total	**1621**		**531**	

A population-based study of genes previously implicated in breast cancer, Chunling Hu *et al*, 2021

6. (a) For each cancer predisposition gene, calculate the percentage of cancer caused by the gene in the table above:

   (b) Calculate the total percentage of both cancer patients and non cancer controls with mutant genes:

   (c) Would you say there is a significant difference between the percentage of both cancer patients and non cancer controls with mutant genes?

   _____

   (d) How could you test for this? _____

7. A person with a family history of breast cancer is tested for the BRCA1 and BRCA2 cancer related alleles and is found to have both. How would you advise this person in relation to their cancer risk and future lifestyle?

   _____

   _____

   _____

   _____

   _____

8. Discuss the use of large scale or population wide studies on gene associations with cancer: _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

106 Population Genetics and Human Evolution

Key Idea: Large scale genetic studies have helped answer questions about how humans evolved and migrated across the globe.

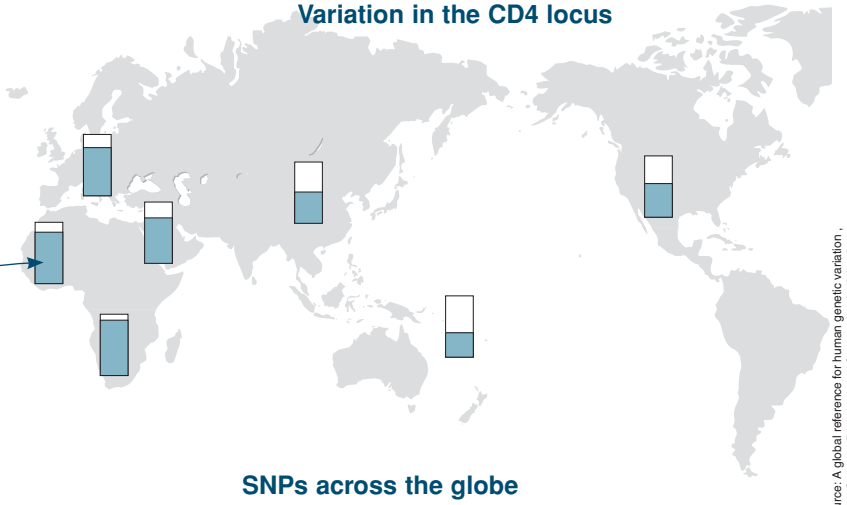
Fossil and genetic evidence shows modern humans evolved around 200,000 years ago in East Africa and then spread across the globe in a series of migrations. The last major migration out of Africa occurred around 70,000 to 80,000 years ago. As humans migrated out of Africa they encountered and interbred with small populations of Neanderthals and Denisovans. Interbreeding with these groups has left traceable amounts of DNA in the genome. The amount varies from region to region across the globe.

Out of Africa

- One of the great debates over human evolution is: did modern humans evolve in Africa and then migrate across the globe (the Out-of-Africa hypothesis), or did they evolve from an earlier migration of *Homo erectus* which produced populations throughout the Old World that evolved together as they interbred (the multiregional hypothesis)?
- Genetic evidence from studies involving groups across the globe shows that genetic diversity is greatest in Africa and becomes less so outside Africa. This is consistent with a subset of humans migrating out of Africa and populating the globe (right).
- Humans, in general, have very little genetic diversity. An average person's DNA varies about 0.6% from the 2015 reference genome.

	Diversity (0 = low)		
Locus (measurement)	Africa	Europe	Asia
30 microsatellites	0.807	0.730	0.685
Xq 13.3 (short arm X chromosome)	0.035	0.034	0.025
50 autosomal sequences	0.115	0.064	0.061
mt DNA control region	2.08	1.08	1.75

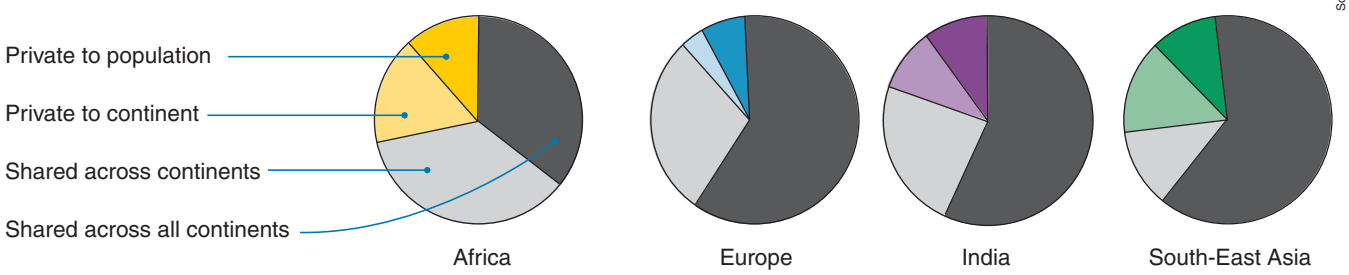
Variation in the CD4 locus



Diversity in the CD4 locus on chromosome 12. (Blue shading represents the amount of diversity).

Source: A global reference for human genetic variation , 1000 Genome Project, 2015. See credits for full reference.

SNPs across the globe



1. Describe the Out of Africa and multiregional hypotheses: _____

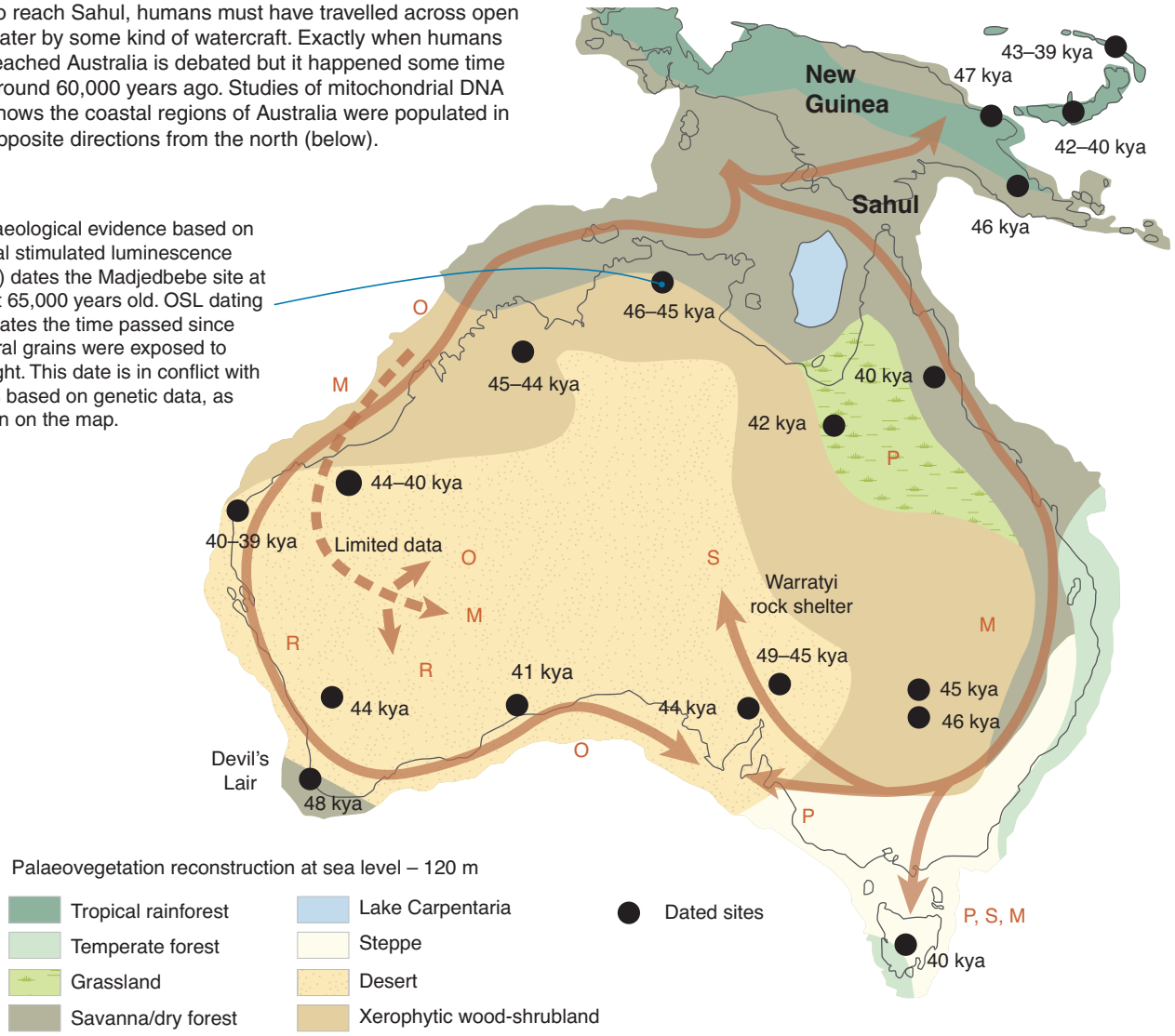
2. Which hypothesis does the genetic evidence support and why? _____

ISBN: 978-1-98-856655-9

Using haplotypes to track the populating of Australia

- After it split from Gondwana, the Australian continent (technically known as Sahul) has remained disconnected from the rest of the world. Low sea levels 70–60,000 years ago saw a land bridge form between mainland Australia and the island of New Guinea to the north, but there has been no connection of Sahul to the other continents.
- To reach Sahul, humans must have travelled across open water by some kind of watercraft. Exactly when humans reached Australia is debated but it happened some time around 60,000 years ago. Studies of mitochondrial DNA shows the coastal regions of Australia were populated in opposite directions from the north (below).

Archaeological evidence based on optical stimulated luminescence (OSL) dates the Madjedbebe site at about 65,000 years old. OSL dating estimates the time passed since mineral grains were exposed to sunlight. This date is in conflict with dates based on genetic data, as shown on the map.



Adapted from Tobler *et al* Nature, 2017. See credits for full reference.

- The latest and most comprehensive mitochondrial DNA analysis has shown the modern Aboriginal Australians are directly related to the first humans to populate Australia. Analysis of Aboriginal mitochondrial haplotypes show they fall into five major groups labelled S, O, M, P and R. The movement of these groups is shown on the map above.
- Results are consistent with the hypothesis that a wave of modern humans migrated out of Africa 80–70,000 years ago. Some of those humans arrived in Australia around 60,000 years ago. Interestingly, Aboriginal Australians diverged from Papuans about 40,000 years ago, long before the physical separation of the two countries 10,000 years ago.
- The diagram above shows the estimated routes taken during the populating of Sahul based on the five mitochondrial haplotypes.

3. Describe the route taken in the populating of Australia: _____

_____

4. Looking at the dates on the map, what can be said about the rate at which Australia was populated?

_____

5. Looking at the locations where the major haplotypes are found, what can be said about migration within Australia after the initial population event?

_____

_____

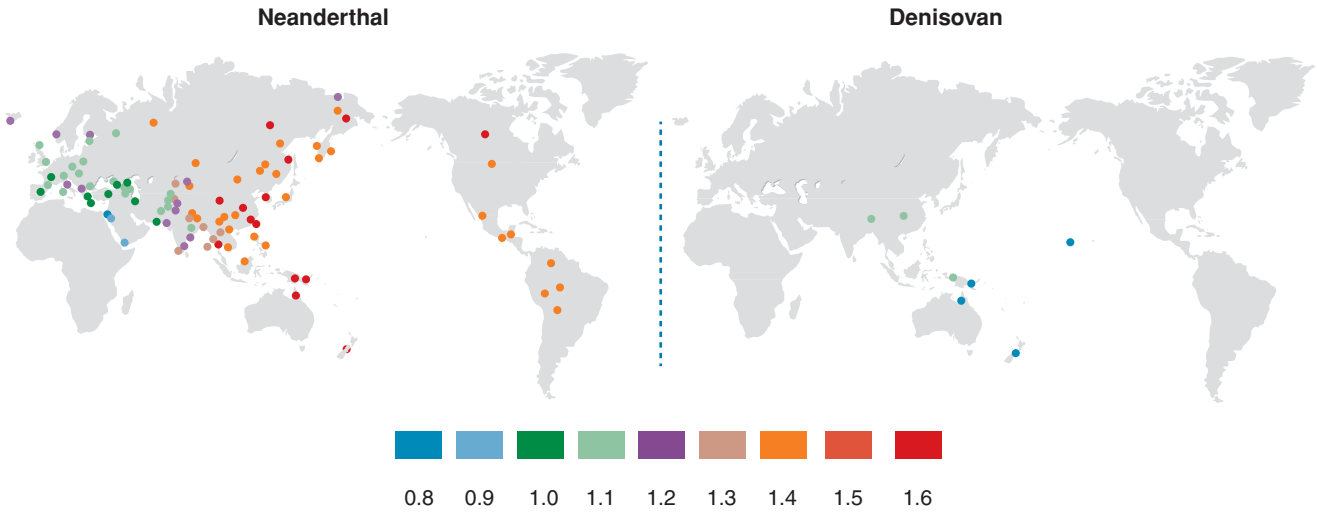
_____

ISBN: 978-1-98-856655-9

Large scale DNA analysis of human groups shows interbreeding with Neanderthals and Denisovans

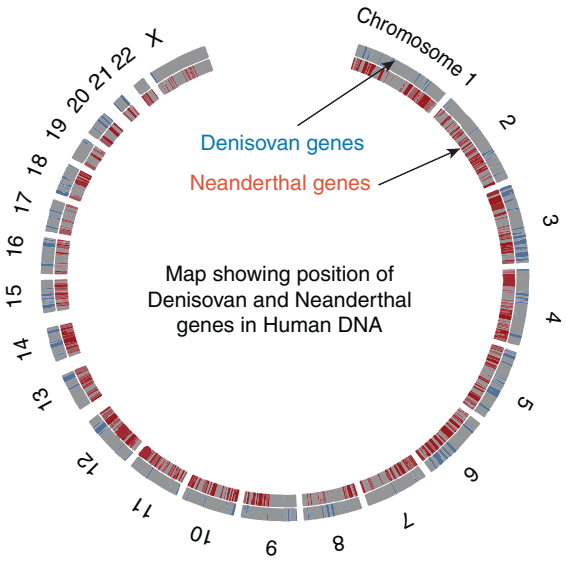
- As modern humans migrated out of Africa they encountered populations of other *Homo* species that had evolved from earlier migrations of *Homo heidelbergensis* out of Africa about 650,000 years ago. These populations were the Neanderthals in Europe and the Denisovans in Asia.
- Until around the late 2000s it was generally assumed that modern humans simply out-competed the Neanderthals, if they met at all. However, when the first Neanderthal DNA was sequenced in 2010 it hinted at a more complex relationship. In 2016, analysis of Neanderthal and human DNA showed that about 2% of the human genome was made up of Neanderthal genes. Further analysis has shown that interbreeding occurred a number of times.
- In 2010, analysis of mtDNA from a tooth found in the Denisova cave in Siberia showed the tooth was from a previously unknown *Homo* species, neither human nor Neanderthal. Comparison of Denisovan and human DNA shows that Denisovan DNA appears in the DNA of peoples of Oceania and Malaysia. Denisovan DNA does not appear in peoples of Europe and Africa.

Percentage Neanderthals or Denisovan DNA in modern human groups



Percentage Neanderthal or Denisovan DNA in modern human groups

Based on data from The landscape of Neanderthal ancestry in present-day humans. Sriram Sankararaman *et al*, 2014



- Denisovan and Neanderthal DNA is spread throughout the human genome. Denisovan DNA appears in larger chunks than Neanderthal DNA, indicating a more recent interbreeding event.
- A recent study on a 45,000 year old human leg bone found in Siberia showed it had 2.1% Neanderthal DNA, the same as a human today. However, the DNA was in large chunks rather than dispersed as it is in our modern genome.
- This indicates the interbreeding event with Neanderthals had recently occurred. Using this as a marker, scientists were able to date the migration out of Africa by modern humans at around 55,000 years ago.

Neanderthal skull

Image: Bone clones

6. Explain how the presence of Neanderthal and Denisovan DNA in modern humans agrees with the theory of a migration out of Africa by modern humans, around 70,000 years ago.

ISBN: 978-1-98-856655-9

107 Chapter Review: Did You Get It?

1. The electrophoresis gel (below, right) shows four profiles containing five STR sites: the mother (A) her daughter (B) and two possible fathers (C and D). Which of the possible fathers is the biological father?

   (a) The biological father is: _____

   (b) Why do profiles B and D only have 9 bands?

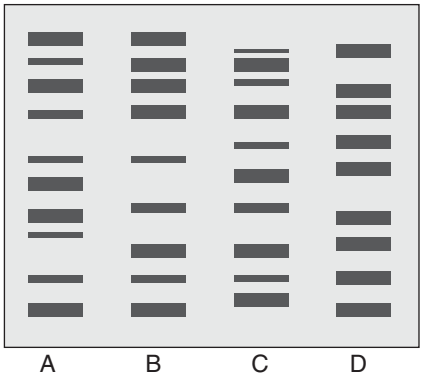
   _____

   _____

   _____

   _____

   _____



2. Distinguish between VNTRs and STRs and outline their uses in modern genetics: _____

   _____

   _____

   _____

   _____

3. Below is a DNA sequence of sections A, B, C, D, E, F and G and A', B', C', D', E', F' and G'. You want to isolate the sections B, C, D and E as a continuous group by PCR (and B', C', D', E'). Primers are E' and B. How many PCR cycles are needed to fully isolate the sequence B, C, D, and E, and B', C', D', and E'?

A	B	C	D	E	F	G
A'	B'	C'	D'	E'	F'	G'

   _____

4. Describe the genetic variation of humans across the globe. What does it show about early human migration?

   _____

   _____

   _____

5. Describe the use of haplotypes in studying the migration of humans across the globe:

   _____

   _____

   _____

6. Test your vocabulary by matching each term to its correct definition, as identified by its preceding letter code:

DNA profiling

forensics

gel electrophoresis

polymerase chain reaction

A A process that is used to separate different lengths of DNA by placing them in a gel matrix placed in a buffered solution through which an electric current is passed.

B A reaction that is used to amplify fragments of DNA using cycles of heating and cooling.

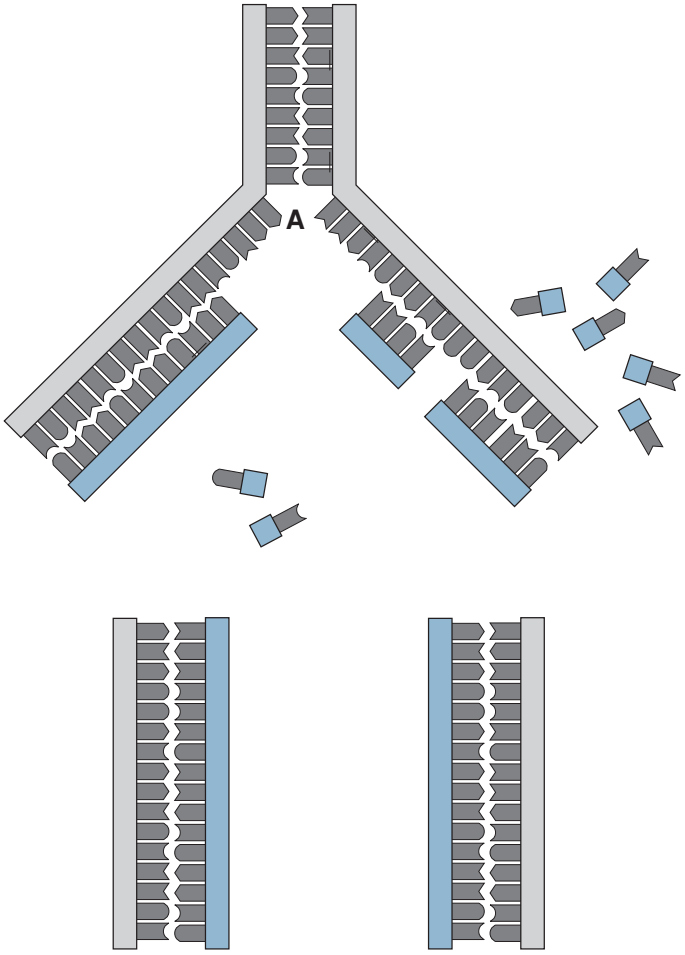
C The application of scientific methods to the investigation of matters involving criminal and civil laws.

D The process of locating regions of a DNA sequence that are variable between individuals in order to distinguish between individuals.

ISBN: 978-1-98-856655-9

108 Synoptic Assessment: Module 5

1. The diagram below shows a simplified overview of DNA replication. Summarise the process by labelling the diagram according to the questions below:



(a) Label the 5' and 3' ends of all strands on the DNA being replicated.

(b) Label:
- (i) A parent strand
- (ii) A daughter strand
- (iii) Free nucleotides
- (iv) The new chromatids
- (v) The leading strand
- (vi) The lagging strand

(c) Draw in the likely positions of:

- (i) DNA polymerase
- (ii) Helicase

(d) What is happening at position A?

_____

_____

_____

_____

(e) Circle the nucleotide to be added next to the leading strand and use an arrow to show where it will go.

(f) Circle the nucleotide to be added next to the lagging strand and use an arrow to show where it will go.

2. Some animals and most plants are able to reproduce asexually.

(a) What is asexual reproduction? _____

_____

_____

(b) Describe a method of asexual reproduction for a named animal, and explain how asexual reproduction is advantageous for the animal:

_____

_____

_____

_____

_____

_____

3. Briefly describe the process of binary fission in prokaryotes: _____

_____

_____

_____

ISBN: 978-1-98-856655-9

4. Meiosis is a type of cell division in sexually reproducing organisms:

   (a) What is the purpose of meiosis? _____

   _____

   (b) How is meiosis different from mitosis? _____

   _____

   _____

   (c) How does meiosis increase the variation in the gametes? _____

   _____

   _____

   _____

   _____

5. In guinea pigs, black hair (B) is dominant over white hair (b), and short hair (L) is dominant over long hair (l). In order to produce consistent and reliable coat colour in guinea pigs, breeders use true breeding parents. A breeder has a male guinea pig she knows to be BBLL, and a female she knows to be bbll. She wants to use them to begin a breeding programme to produce a pure breeding, white, short haired guinea pig.

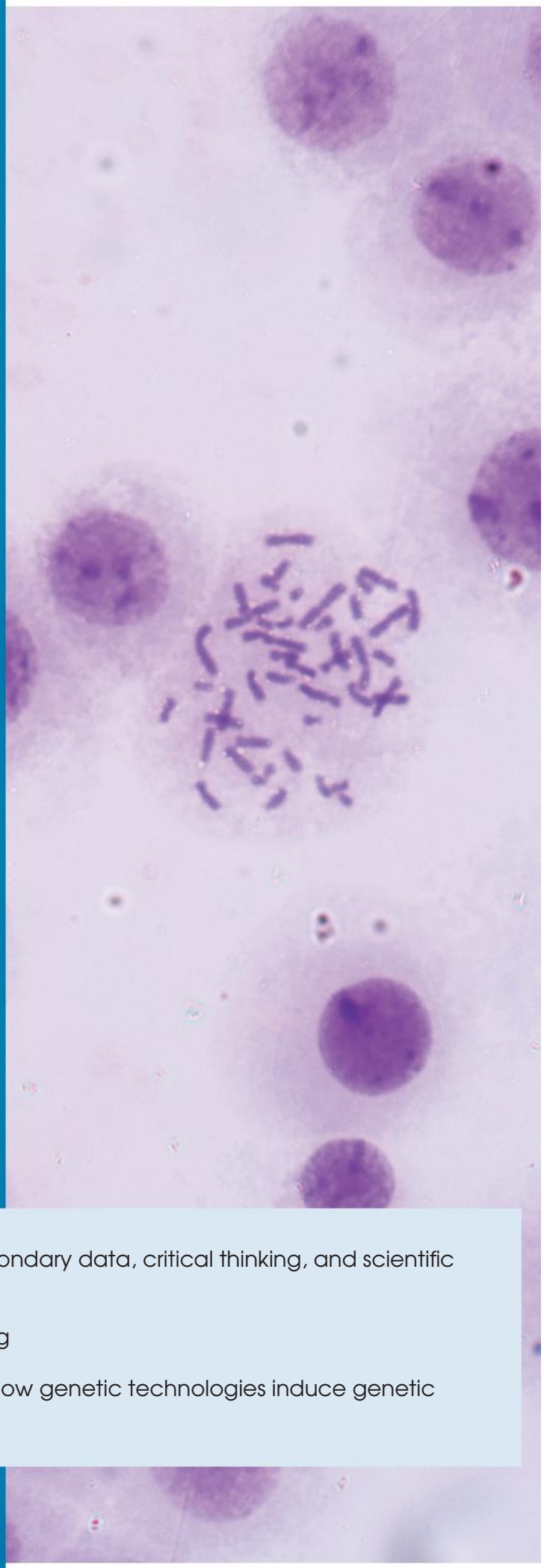
   Explain how the desired pure breeding animal can be produced, and how its ability to breed true can be proved. Support your answer with Punnett squares, and the correct use of the allele letters given (you may use extra paper if required):

ISBN: 978-1-98-856655-9

MODULE

06

Genetic Change



Student outcomes:

- Solve problems using primary and secondary data, critical thinking, and scientific processes
- Communicate scientific understanding
- Explain natural genetic change and how genetic technologies induce genetic change

CHAPTER 6

Mutation

Key terms

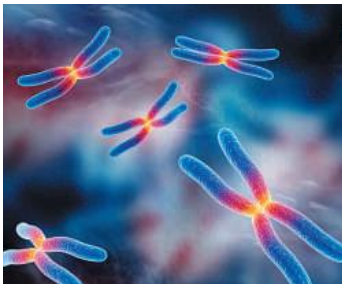
aneuploidy
code degeneracy
coding DNA
deletion
duplication
exon
frameshift mutation
germ-line
insertion
intron
inversion
missense mutation
mutagen
mutation
non-coding DNA
nonsense mutation
polyploidy
silent mutation
somatic
substitution
translocation

Activity number

Inquiry question: *How does mutation introduce new alleles into a population?*

Mutation as a source of variation

Key skills and knowledge



The effect of mutation on population gene pools

Key skills and knowledge

109 Mutagens

Key Idea: Mutagens are chemical or physical agents that cause a change in the DNA sequence.

Mutations occur spontaneously in all organisms. The natural rate at which a gene will undergo change is normally very low, but this rate can be increased by environmental factors such as ionising radiation and mutagenic chemicals.

Only mutations in sex cells (**germ-line mutations**) will be inherited. If mutations occur in a body cell after the organism has begun to develop, they are called **somatic mutations**. In some cases, somatic mutations may disrupt the normal controls over gene regulation and expression, and trigger the onset of **cancer** (abnormal cell growth).

Mutagens and effects

Ionising radiation

High energy radiation in the form of ultraviolet radiation, x-rays, gamma rays and particle emission from radioactive isotopes can penetrate tissue and cause DNA damage. Rates of thyroid cancer increased in areas near Chernobyl after the explosion of the No. 4 reactor there. Skin cancer from high exposure to ultraviolet is increasingly common and fair skinned people at low latitudes are at greatest risk. Safer equipment has reduced the risks to those working with ionising radiation, e.g. radiographers.

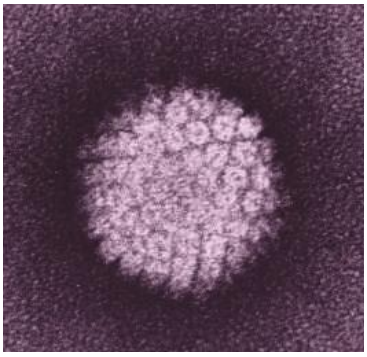
Chernobyl No. 4 reactor after the explosion in 1986

Soviet Authorities Fair Use

Viruses and microorganisms

Some viruses integrate into the human chromosome, upsetting genes and triggering cancers. Examples include hepatitis B virus (liver cancer), HIV (Kaposi's sarcoma), Epstein-Barr virus (Burkitt's lymphoma, Hodgkin's disease), and HPV (right) which is implicated in cervical cancer. Aflatoxins produced by the fungus *Aspergillus flavus* are potent inducers of liver cancer.

Those at higher risk of viral infections include intravenous drug users and those with unsafe sex practices



NIH

Poisons and irritants

Many chemicals interact directly with DNA to trigger cancer (they are carcinogenic). Synthetic and natural examples include organic solvents, e.g. benzene, tobacco tar, formaldehyde, vinyl chlorides, coal tars, some dyes, and nitrites. Those most at risk include workers in the chemicals industries, including the glue, paint, rubber, resin, and leather industries, petrol pump attendants, and those in the coal and other mining industries.

Right: Firefighters and those involved in environmental clean-up of toxic spills are at high risk of exposure to mutagens.

Diet, alcohol and tobacco

Diets high in fat, especially fatty, highly preserved meat, slow the passage of food through the gut, giving time for mutagenic irritants to form in the lower bowel.

High alcohol intake increases the risk of some cancers and increases susceptibility to tobacco-smoking related cancers.

Tobacco tars contain at least 17 known carcinogens (cancer inducing mutagens) that cause chronic irritation of the gas exchange system and cause cancer in smokers.

1. Describe examples of environmental factors that induce mutations under the following headings:

   (a) Ionising radiation: _____

   _____

   _____

   _____

   _____

   _____

   (b) Chemical agents: _____

   _____

   _____

   _____

   _____

   _____

2. Explain how mutagens cause mutations:

   _____

   _____

   _____

   _____

   _____

   _____

3. How might a high fat diet contribute to mutations that could cause cancer?

   _____

   _____

   _____

   _____

   _____

   _____

   _____

   _____

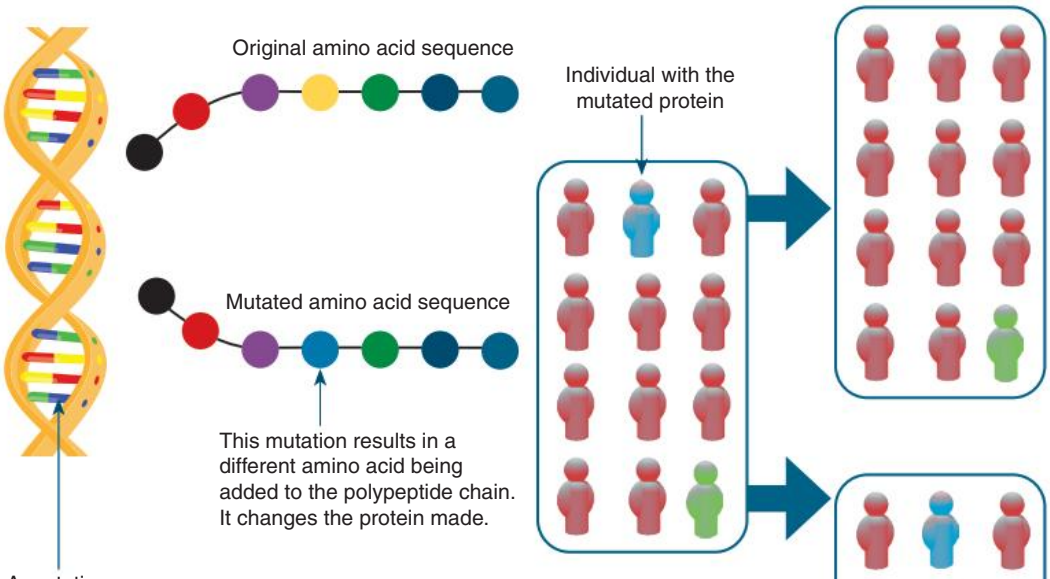
ISBN: 978-1-98-856655-9

110 Mutations

Key Idea: Mutations are changes to the DNA sequence and result in new alleles. Most mutations are harmful, but occasionally they are beneficial.

Mutations can be small, such the change of a single nucleotide (e.g. A to G) or they may be large, such as the duplication of entire genes or sometimes entire genomes. Most mutations are harmful because they disrupt some important cellular process, often by causing a protein to fold incorrectly. Occasionally, they may cause some beneficial change, such as making an enzyme more efficient. Although the DNA replication process is very accurate, it is estimated that, in humans, a mutation occurs once every 30 million base pairs copied during DNA replication prior to meiosis. This means that everyone has about 200-300 new mutations that their parents did not have. In most cases, these mutations have little to no effect and are called silent mutations.

Some mutations are retained, others are eliminated



If the mutation is harmful (reduces fitness) in the current environment, it is selected against and is usually eliminated from the population.

If the mutation is beneficial (increases fitness) **and** it is heritable (occurs in the gametes) it is selected for and retained in the population. It may become more common over several generations.

A silent mutation is a DNA sequence change that has no phenotypic effect. This may be because the change occurs outside a protein-coding region (in introns), because there is no change in the amino acid (due to code degeneracy) or because an amino acid has been replaced by one with the same properties. Heritable silent mutations may be carried without effect and may only be subject to selection pressure when environmental conditions change.

DID YOU KNOW?

There is evidence that some 'silent' changes affect mRNA stability and transcription, even though they do not change codon information. In these cases, the mutations are not neutral.

1. What is a mutation? _____

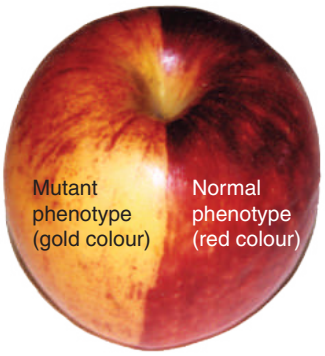
2. Why are some mutations retained within a population and others eliminated? _____

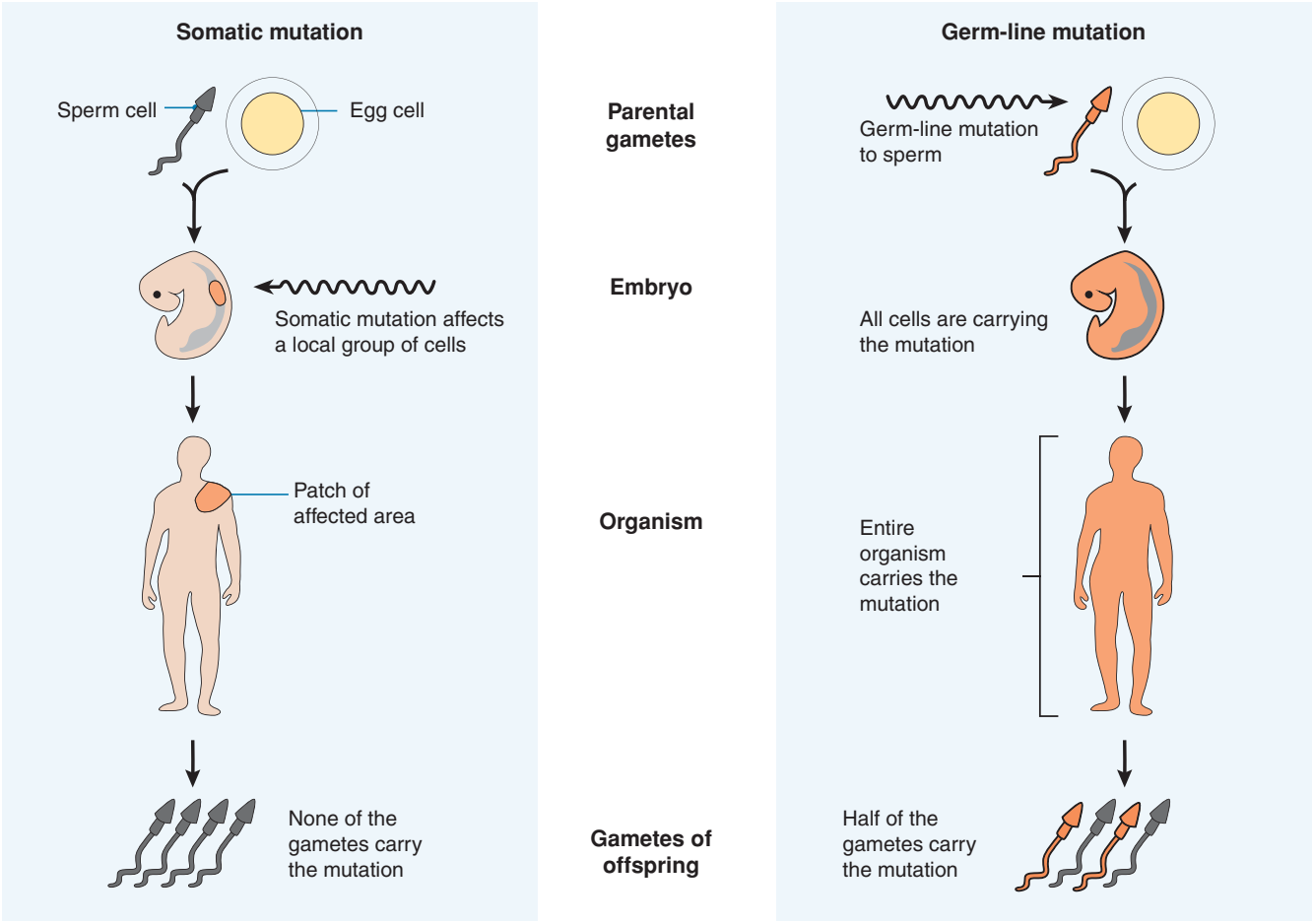
3. (a) What is a silent mutation? _____

   (b) Explain the potential advantage of a silent mutation being retained within a population? _____

ISBN: 978-1-98-856655-9

- Gametic cells are the reproductive (sex) cells of an organism (the egg and sperm). Mutations occurring in these cells are called **germ-line mutations,** or **gametic mutations**.
- Somatic cells (body cells) are all the remaining cells. Mutations to these cells are called **somatic mutations**.
- Only germ-line mutations will be inherited. Somatic mutations are not inherited but may affect an organism in its lifetime (e.g. a cancer).
- The red delicious apple (right) is a natural chimera (an organism with a mixture of two or more different genotypes). In the apple, a mutation occurred in the part of the flower that developed into the fleshy part of the apple. The seeds are unaffected by the mutation, so it is not inherited.





4. Distinguish between somatic and germ-line mutations: _____

5. Explain the consequences of these different mutation locations: _____

6. Chimeras can be produced artificially in both plants and animals. What kind of information could these organisms provide in studies of gene expression and gene regulation?

ISBN: 978-1-98-856655-9

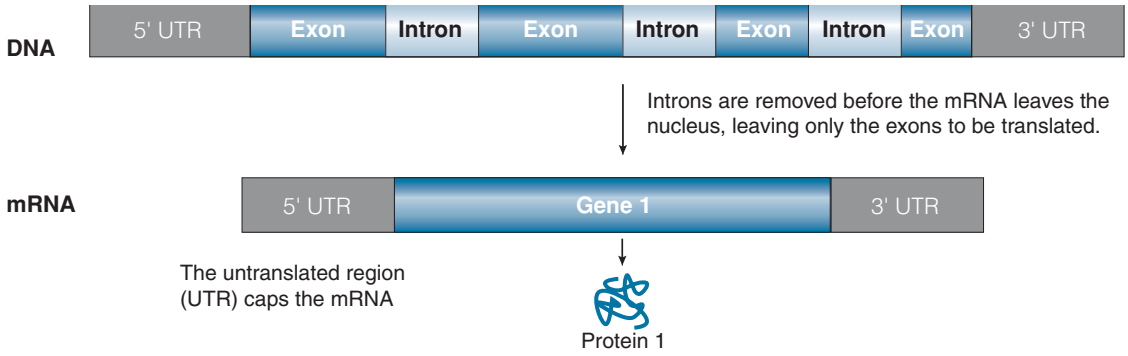
Coding and non-coding DNA

Most of a cell's DNA does not code for polypeptides. Some of this non-coding DNA has other functions within the cell and mutations within these areas can be harmful.

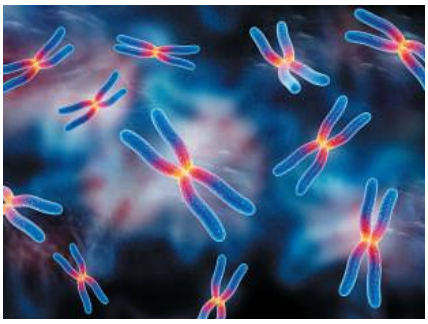
- Regions of coding DNA, **exons**, are used to make polypeptides. Mutations in the coding DNA can result in polypeptides that do not function properly.
- Non-coding DNA, or **introns**, can have other regulatory roles such as controlling the amount and frequency of polypeptide produced. Any mutation in these areas can therefore affect gene regulation.
- Some other non-coding DNA makes up the chromosome structure, including telomeres and centromeres. Any mutations in these areas can affect the functions of these structures.

Non-coding DNA within genes

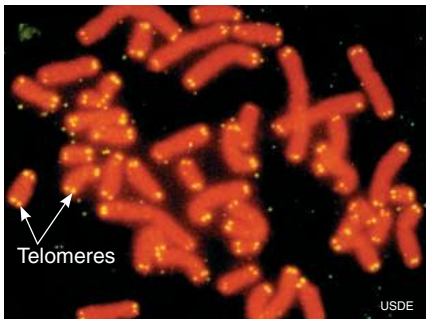
- Within genes, there are stretches of coding DNA known as exons and non-coding DNA called introns
- Before the primary RNA transcript is translated in eukaryotes, the non-protein coding introns are removed. Only the protein-coding exons form the mature mRNA for translation.



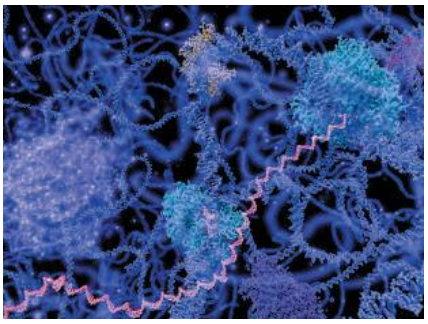
Other non-coding DNA



Centromeres are non-coding DNA sequences. During meiosis, spindle fibres attach to the chromatids and pull the chromatids apart. Mutations to these regions are often found in cancer cells.



Telomeres are repeating, non-coding DNA sequences found at the end of chromosomes. They protect the chromosomes from degradation during replication.



Regulatory genes (elements/sequences) are sections of DNA that regulate protein-coding genes. They include promoters and enhancers, that facilitate transcription of genes.

7. What effect might a mutation in a gene regulator section of non-coding DNA have?

8. How might a mutation in a section of DNA that makes up a centromere affect the cell? _____

9. Describe two functions of non-protein coding DNA: _____

ISBN: 978-1-98-856655-9

111 Types of Gene Mutations

Key Idea: Gene mutations are localised changes to the DNA base sequence. In most cases this will cause a change in phenotype.

Gene mutations are small, localised changes in the DNA base sequence caused by a mutagen, or an error during DNA replication. The changes may involve a single nucleotide (a **point mutation**) or a triplet. Point mutations can occur by **substitution**, **insertion**, or **deletion** of bases and alter the mRNA transcribed. A point mutation may not alter the amino acid sequence because more than one codon can code for the same amino acid; this is called **code degeneracy**. Such mutations are often called silent because the change is not recorded in the amino acid sequence. Mutations that cause a change in the amino acid sequence may be harmful because they alter protein function, but may occasionally be beneficial in the right selective environment.

Normal gene sequence

The DNA sequence below is a **coding strand** sequence. It codes for a hypothetical polypeptide (right) that makes a pigment, affecting skin tone. If the polypeptide is correctly translated from the DNA, skin tone will be dark. An incorrectly translated polypeptide will produce a different skin tone. We can compare the normal gene sequence (below) and the normal polypeptide (right) to examine the effect of various point mutations in the following examples.

1 Ala

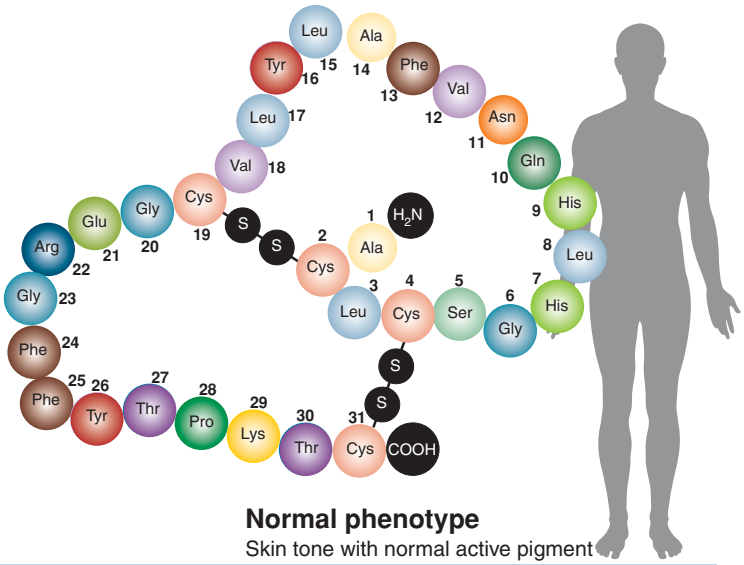
GCT TGT TTA TGC TCT GGC CAT CTT CAC CAA AAT

GTT TTT GCT CTG TAT CTG GTT TGT GGT GAA CGT

GGG TTT TTT TAT ACT CCC AAA ACT TGT

Cys 31

Normal polypeptide chain



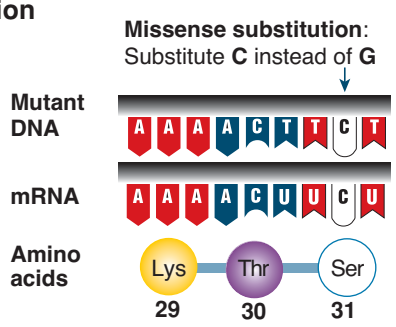
Normal phenotype
Skin tone with normal active pigment

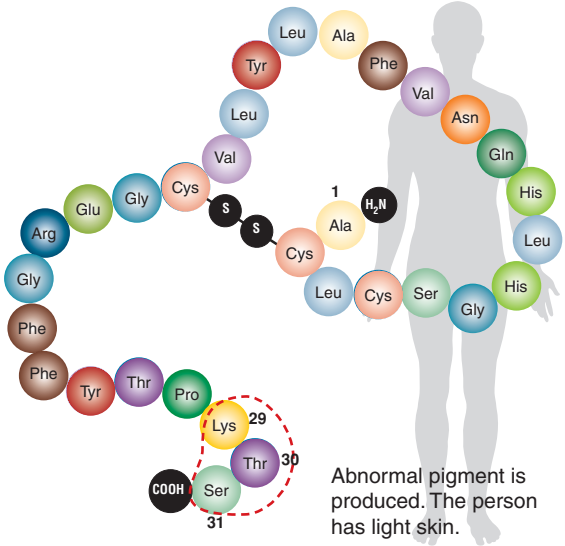
Substitution mutations

In a substitution mutation a single base is substituted with another. Some substitutions may still code for the same amino acid (silent mutation) because of code degeneracy, but they may also result in a codon that codes for a different amino acid.

Missense substitution

In this example, a substitution mutation has altered the 31st triplet TGT to TCT. This results in the amino acid serine appearing where cysteine should be. This **missense** could affect the protein's function.

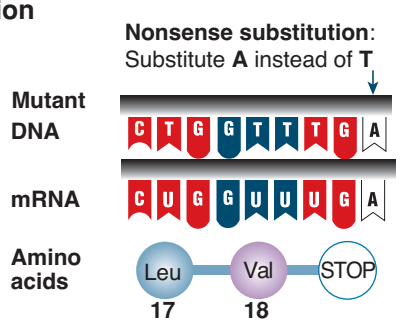


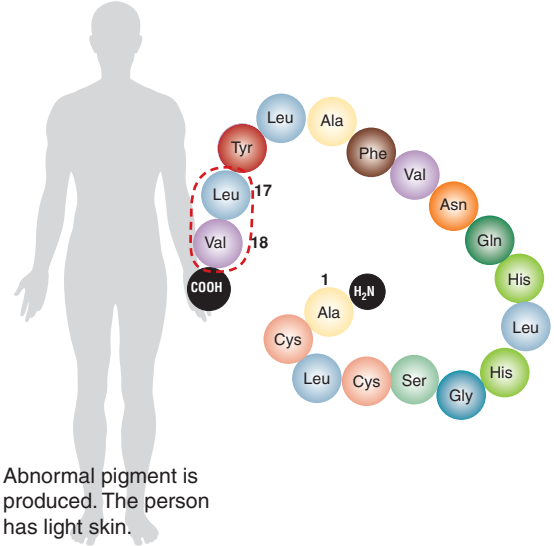


Abnormal pigment is produced. The person has light skin.

Nonsense substitution

In this example, the change from TGT to TGA at the 19th codon translates as a stop signal. When the mRNA is translated, it ends prematurely, leaving a shortened protein. This is a **nonsense** substitution.



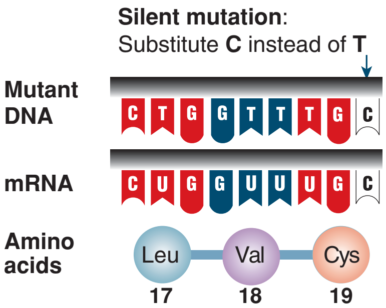


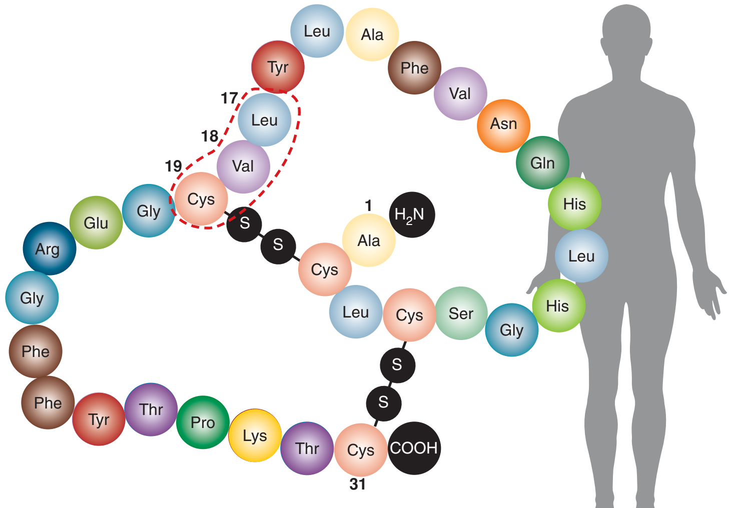
Abnormal pigment is produced. The person has light skin.

ISBN: 978-1-98-856655-9

Silent mutations

Silent mutations do not change the amino acid sequence nor the final protein because several codons may code for the same amino acid. In the example (right), a substitution on the 19th triplet alters the TGT to TGC. The triplet still codes for cysteine, so the pigment is still functional. Silent mutations are not always neutral as they may affect mRNA stability and transcription, even though they do not change codon information.

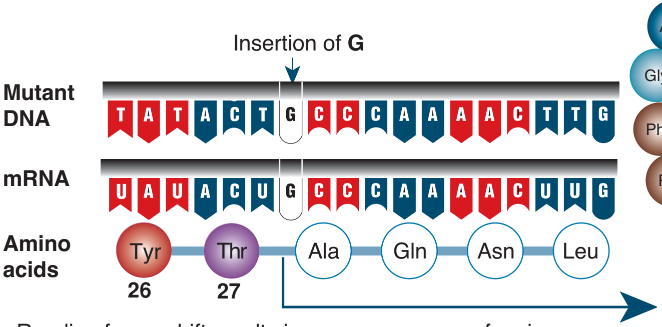




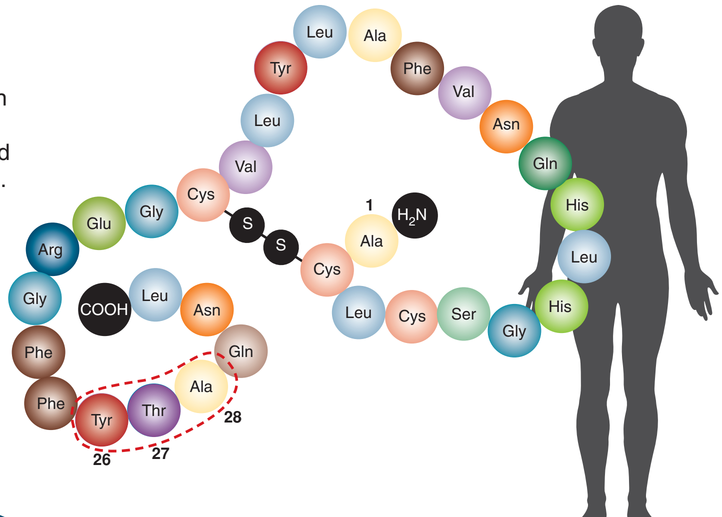
Skin tone with normal active pigment.

Insertion mutation

An insertion mutation involves the addition (insertion) of an additional base into the DNA sequence. The insertion of a single extra base displaces the bases after the insertion by one position (called a **reading frame shift**). In the example (right), a G is inserted after the DNA triplet at position 27. This results in altered amino acids and may cause loss or, in this case, gain of function.



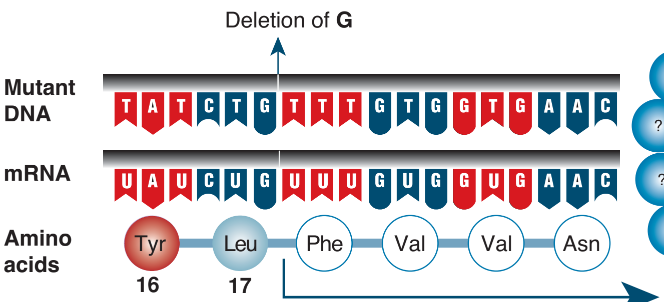
Reading frame shift results in a new sequence of amino acids. The protein could have altered or no biological activity.



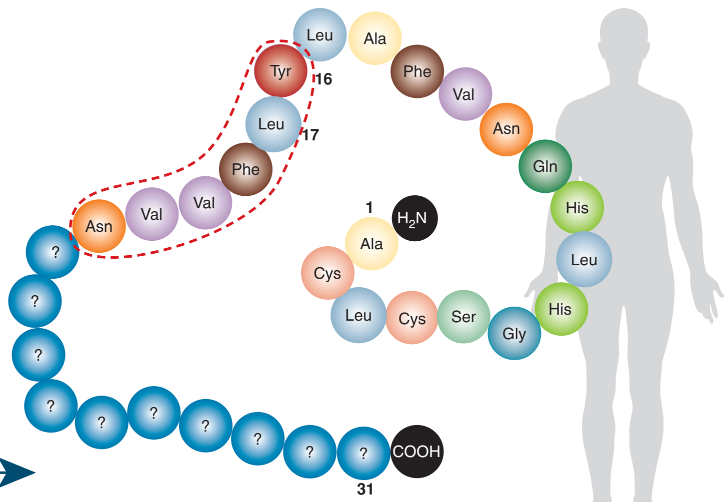
The new sequence produces a different shape in the pigment that makes it darker. This is advantageous in high UV environments.

Deletion mutation

The deletion of a nucleotide is called a **deletion mutation**. It also causes a reading frame shift. In this example the nucleotide G is deleted after the 17th DNA triplet. This leads to a reading frame shift that affects the rest of the protein. This could lead to nonsense.



Reading frame shift results in a new sequence of amino acids. The protein could have altered or no biological activity.



Abnormal pigment. The person has light skin.

ISBN: 978-1-98-856655-9

1. Why are mutations that alter the amino acid sequence usually harmful? _____

2. Why are nonsense substitutions likely to be more damaging than missense substitutions? _____

3. Explain how a substitution mutation can be effectively silent: _____

4. What is a reading frame shift?_____

5. Why is a frame shift near the start codon likely to have a greater impact than one near the stop codon? _____

6. (a) Use the mRNA table on page 89 to fill in the missing amino acids from position 22 to 31 on the deletion mutation protein on the preceding page. The mutant DNA sequence in given below to help you:

   Mutant DNA (coding strand): GTG GGT TTT TTT ATA CTC CCA AAA CTT GT?

   Mutant mRNA: _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

   Amino acids: _____ _____ _____ _____ _____ _____ _____ _____ _____

   (b) Decide how affected the protein would be by this change in amino acids and justify your decision:

7. Discuss how point mutations can affect the phenotype of an organism. Use the example of the hypothetical skin pigment to illustrate your points:

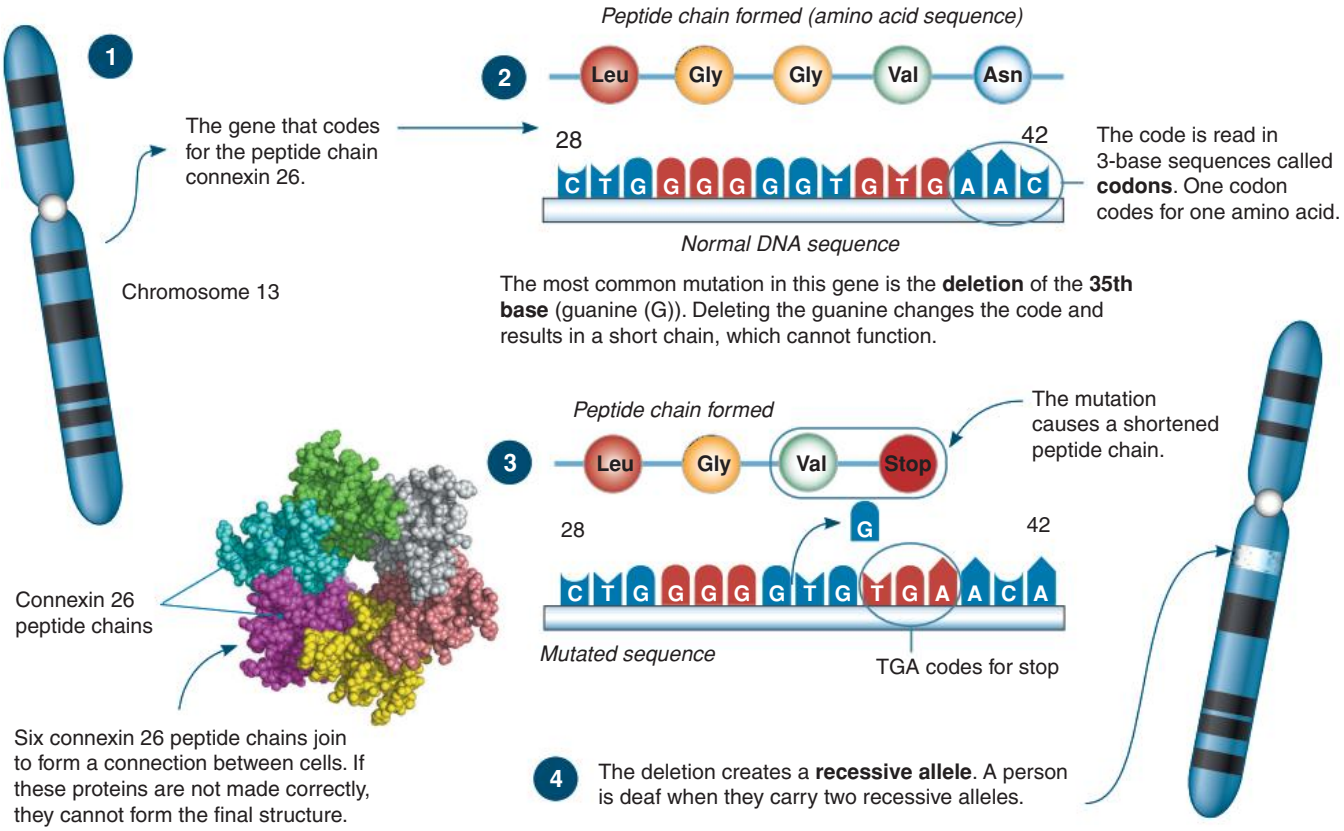
ISBN: 978-1-98-856655-9

112 Examples of Gene Mutations

Key Idea: Mutations are the only way to create new alleles, They are usually harmful but can occasionally be beneficial.

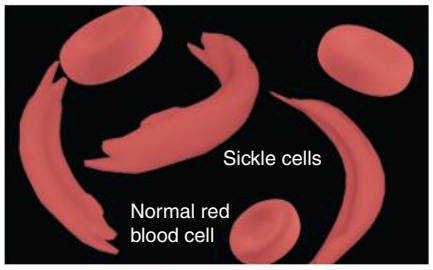
Mutations are changes to the DNA sequence. They may involve small changes to the DNA, e.g. a base substitution, or movements of large parts of chromosomes. Sometimes mutations are silent and result in no change in the phenotype (appearance) of the individual. The example below describes a mutation that produces a new allele for the gene coding for the protein connexin 26. This mutation causes a form of inherited deafness (NSRD).

Mutation for non-syndromic recessive deafness (NSRD)



In mammals, production of the enzyme lactase, which digests the sugar lactose in milk, stops after childhood. In humans of European, East African, or Indian descent, a mutation about 10,000 years ago produced a dominant allele for the lactase gene. This kept the lactase gene active, allowing adults to continue to digest dairy products.

In humans, there are three major blood groups, A, B, and O, controlled by the alleles A, B, and O. The alleles produce enzymes that modify carbohydrates (sugars) on the surface of blood cells. Different blood groups have different carbohydrates on the cell surface. It is believed that the A allele group evolved first, followed by O, and then B.



Red blood cells are packed with the oxygen-carrying protein haemoglobin, encoded by the HBB gene. A substitution mutation to the HBB gene produces an allele that causes the haemoglobin to distort the red blood cells into a sickle shape. The homozygous condition is lethal but the mutation persists because heterozygotes are more resistant to malaria.

1. The NSRD mutation is a harmful mutation. Why might someone with this mutation not actually be affected?

2. Why would the appearance of the persistent lactase allele be an advantage? _____

ISBN: 978-1-98-856655-9

113 Inherited Metabolic Disorders

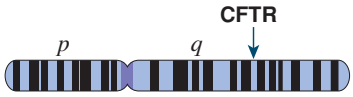
Key Idea: Many genetic diseases in humans are the result of mutations to recessive alleles, but some are also caused by dominant or codominant alleles.

There are more than 6000 human diseases attributed to mutations in single genes, although most are uncommon. The three genetic diseases described below occur with relatively high frequency and are the result of recessive, dominant, and codominant allele mutations respectively.

Cystic fibrosis (CF)

Cystic fibrosis is traditionally treated with physical therapy to clear mucus from the airways.

Incidence: Varies with populations:
United States: 1 in 1000 (0.1%).
Asians in England: 1 in 10,000
European descent: 1 in 20-28 are carriers.
Gene type: Autosomal recessive. The most common mutation is ΔF508, which accounts for around 70% of all defective CF genes. The mutation is a deletion of three bases spanning the 507/508th triplets. The net effect is the loss of the amino acid phenylalanine from the CFTR protein, which normally regulates chloride transport in cells. It is non-functional as a result.
Gene location: Chromosome 7



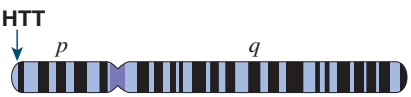
Symptoms: Disruption of all glands including pancreas, bronchial glands (chronic lung infections), and sweat glands (high salt content becomes depleted).
Inheritance: Autosomal recessive pattern. Affected people are homozygous recessive for the mutation. Heterozygotes have largely no symptoms and there is some evidence that they are less susceptible to cholera than people without the mutation.

Huntington disease (HD)

NYWTS

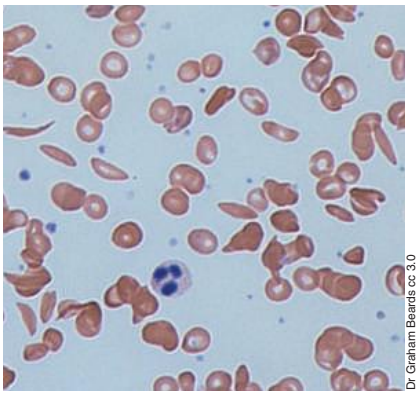
American singer-songwriter and folk musician Woody Guthrie died from complications of Huntington disease.

Incidence: An uncommon disease affecting 3-7 per 100,000 people of European descent. Less common in other ethnicities (e.g. Chinese, Japanese, African).
Gene type: Autosomal dominant mutation of the HTT gene caused by a trinucleotide repeat expansion on the short arm of chromosome 4. In the mutation (**mHTT**), the number of CAG repeats increases from the normal 6-30 to 36-125. The severity of the disease increases with the number of repeats. The repeats result in an abnormally long version of the huntingtin protein.
Gene location: Chromosome 4



Symptoms: The long huntingtin protein is cut into smaller toxic fragments, which accumulate in nerve cells and eventually kill them. The disease becomes apparent in mid-adulthood, with jerky, involuntary movements and loss of memory, reasoning, and personality.
Inheritance: Autosomal dominance pattern. Affected people may be homozygous or heterozygous for the mutant allele.

Sickle cell anaemia



Dr Graham Beards cc 3.0

In a person heterozygous for the sickle cell allele, only some of the red blood cells are deformed.

Incidence: Occurs most commonly in people of African descent.
West Africans: 1% (10-45% are carriers).
West Indians: 0.5%.
Gene type: Autosomal mutation involving substitution of a single nucleotide in the HBB gene that codes for the beta chain of haemoglobin. The allele is codominant. The substitution causes a change in a single amino acid. The mutated haemoglobin behaves differently when deprived of oxygen, causing distortion of the red blood cells, anaemia, and circulatory problems.
Gene location: Chromosome 11

Symptoms: Sickling of red blood cells, which are removed from circulation, anaemia, pain and damage to tissues and organs.
Inheritance: Autosomal codominance pattern. People who are homozygous for the mutant allele have sickle cell disease. Heterozygotes (carriers) are only mildly affected and show greater resistance to malaria than people without the mutation.

1. For each of the genetic disorders below, indicate the following:

   (a) Sickle cell anaemia: Gene name: _____ Chromosome: _____ Mutation type: _____

   (b) Cystic fibrosis: Gene name: _____ Chromosome: _____ Mutation type: _____

   (c) Huntington disease: Gene name: _____ Chromosome: _____ Mutation type: _____

2. Explain why mHTT, which is dominant and lethal, does not disappear from the population: _____

   _____

3. Suggest why the sickle cell mutation has been maintained in populations, despite being lethal: _____

   _____

   _____

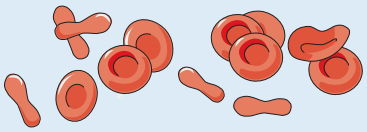
ISBN: 978-1-98-856655-9

114 Sickle Cell Mutation

Key Idea: The substitution of one nucleotide from T to A results in sickle cell disease. The mutation is codominant.

Sickle cell disease, including sickle cell anaemia, is an inherited blood disorder caused by a gene mutation (Hb^s), producing a faulty beta (β) chain haemoglobin (Hb) protein. The disease causes the body to produce red blood cells with a deformed sickle shape and reduced oxygen carrying capacity. Many other aspects of the body's metabolism are also affected. The mutation is codominant (both alleles equally expressed), and people heterozygous for it (carriers) have enough functional haemoglobin such that they suffer only minor effects.

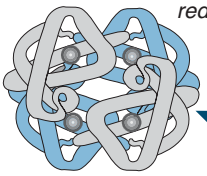
Normal red blood cells



Each red blood cell (RBC) contains about 270 million haemoglobin molecules. In their normal state, the red blood cells have a flattened disc shape which allows them to squeeze through capillaries to offload their oxygen to tissues.

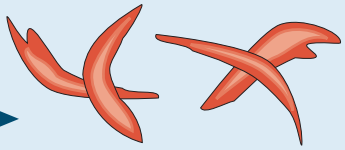
Normal haemoglobin produces normal red blood cells

Mutant haemoglobin produces sickle-shaped red blood cells



Each **haemoglobin molecule** is made up of two α-chains and two β-chains linked together

Sickle cells



The mutated form of haemoglobin has reduced solubility and precipitates when deprived of oxygen. This deforms the red blood cells giving them a rigid sickle shape, which prevents their movement through capillaries.

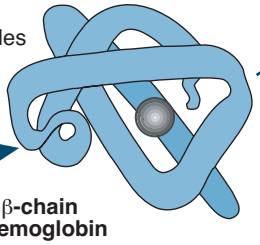
Sickle cell anaemia

The sickled RBCs are removed from the circulation leading to anaemia. Their rigid shape blocks small vessels and leads to widespread tissue and organ damage.

The HBB Gene

The gene coding for the β-chain of haemoglobin is on chromosome 11 and consists of 438 bases.

The 438 nucleotides produce a protein made up of 146 amino acids



β-chain haemoglobin

This sequence is the beginning of the DNA template strand for a normal β-chain of haemoglobin (excluding start sequence TAC). The sickle cell mutation involves the **substitution** of one base for another in the HBB gene, causing one amino acid to be altered. This new amino acid is hydrophobic rather than hydrophilic, which makes the Hb collapse in on itself when deprived of oxygen.

Sickle cell and malaria

The sickle cell mutation (HbS) is lethal in the homozygote (two mutated alleles present) but heterozygotes (one mutated allele) are much less susceptible to malaria than unaffected people (two normal alleles). This is because the malarial parasite cannot infect the deformed blood cells. A high frequency of the mutation is present in many regions where malaria is endemic (present in the population all the time). The ability of a heterozygote to confer an adaptive advantage like this is called **heterozygous advantage**.

1. (a) Explain the genetic cause of sickle cell disease: _____

_____

(b) How does the sickle cell mutation result in the symptoms of the disease? _____

_____

_____

(c) Explain why heterozygotes (carriers) suffer only minor effects: _____

_____

_____

_____

2. Briefly explain why there is a high frequency of the sickle cell mutation in populations where malaria is endemic:

_____

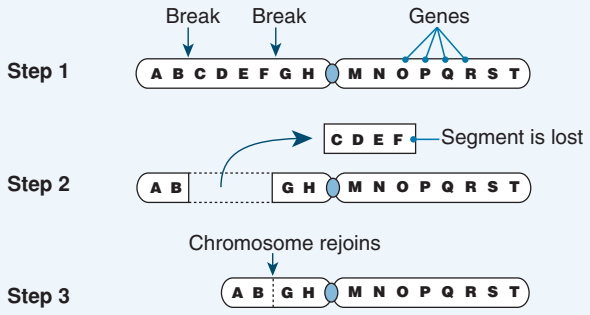
ISBN: 978-1-98-856655-9

115 Chromosome Mutations

Key Idea: Large scale mutations occurring during meiosis can fundamentally change chromosome structure.

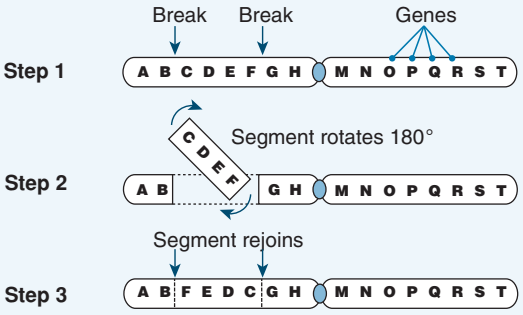
Chromosome mutations, also called block mutations, involve the rearrangement of whole blocks of genes involving many bases, rather than individual bases within a gene. They commonly occur during meiosis and they alter the number or sequence of whole sets of genes on the chromosome (represented by letters below). Translocations may sometimes involve the fusion of whole chromosomes, thereby reducing the chromosome number of an organism. This is thought to be an important evolutionary mechanism by which instant speciation can occur.

Deletion



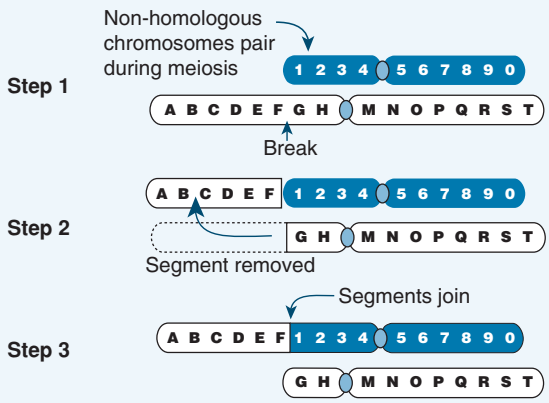
A break may occur at two points on the chromosome and the middle piece of the chromosome falls out. The two ends then rejoin to form a chromosome deficient in some genes. Alternatively, the end of a chromosome may break off and is lost.

Inversion



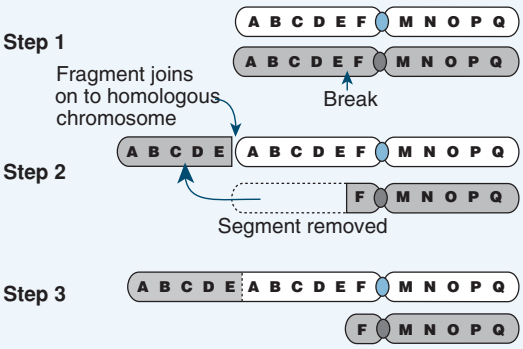
The middle piece of the chromosome falls out and rotates through 180° and then rejoins. There is no loss of genetic material. The genes will be in a reverse order for this segment of the chromosome.

Translocation



In translocation mutations, a group of genes moves between different chromosomes. The large chromosome (white) and the small chromosome (blue) are not homologous. A piece of one chromosome breaks off and joins to the other. When the chromosomes are passed to gametes, some gametes will receive extra genes, while some will be deficient.

Duplication



A segment is lost from one chromosome and is added to its homologue. In this diagram, the darker chromosome on the bottom is the 'donor' of the duplicated piece of chromosome. The chromosome with the segment removed is deficient in genes. Some gametes will receive double the genes while others will have no genes for the affected segment.

1. Which of the chromosome mutations above results in a loss of genetic information? _____

2. For each of the chromosome (block) mutations below, write the new gene sequence after the mutation has occurred:

	Original sequence	Mutated sequence
(a) Inversion:	A B C D E F G H M N O P Q R S T	_____
(b) Translocation:	1 2 3 4 5 6 7 8 9 0	_____

3. Which type of block mutation is likely to be the least damaging to the organism? Explain your answer:

_____

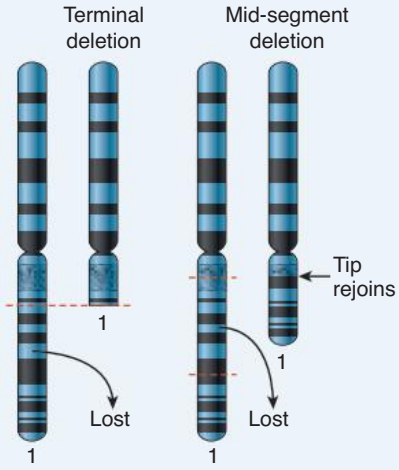
_____

4. Why do translocations sometimes reduce the total number of chromosomes? _____

_____

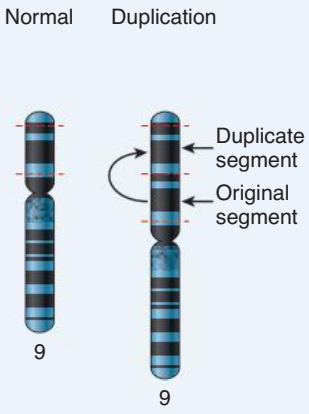
ISBN: 978-1-98-856655-9

Examples of chromosome mutations in humans



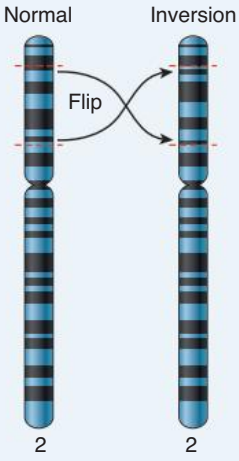
Deletion

Two types of deletion are known to occur on chromosome **1**: a '*terminal deletion*' on the left removes a portion at the end, while an '*mid-segment deletion*' on the right removes a central block of a chromosome.



Duplication

In this chromosome **9** duplication mutation, the extra chromosomal segment could arise via an unequal exchange of chromatids during crossing over with its homologue.



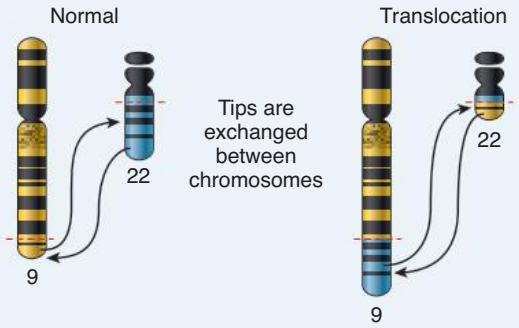
Inversion

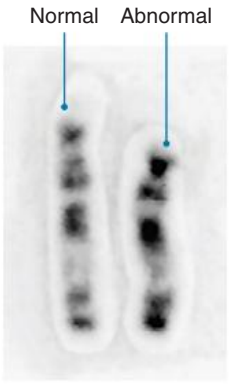
This diagram shows a chromosome **2** inversion. There has been no change in the genetic content; it has only been rearranged. However, this has important consequences during crossing over.

Translocation

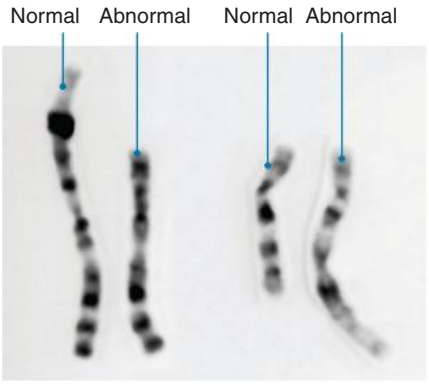
The chromosome on the right shows a two-way translocation where the tips of chromosomes **9** and **22** are exchanged. This specific translocation is associated with **chronic myeloid leukaemia**.

This translocation is a vivid demonstration that if the stability of the chromosome structure is upset too greatly, the resulting phenotype may have impaired fitness (reduced ability to survive and reproduce). This example did not even involve the loss of genetic material, just a movement of genes from one chromosome to another. It may be that an effect on a specific gene at the point of the break caused the illness.

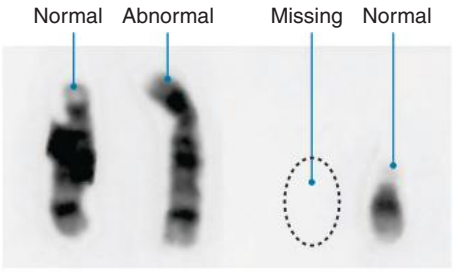




Chromosome 15



Chromosome 1 **Chromosome 10**



Chromosome 14 **Chromosome 21**

Offspring inheriting two normal chromosome 21s as well as the abnormal chromosome 14 have a rare type of Down syndrome (they have three copies of the genes on chromosome 21).

The examples above illustrate block mutations in human chromosomes. Individuals with these karyotypes could produce faulty gametes, leading to recognisable genetic disorders in the offspring. Individuals with translocated chromosomes could produce normal gametes but also gametes either missing or with extra genetic material. *Photos and information: Cytogenetics Department, Waikato Hospital, Hamilton, NZ.*

5. The photographs above were taken by the cytogenetics department at a hospital. Using the diagram explanations on this page as a guide, draw arrows to show the movement of chromosome segments on the photographs.

6. Identify the type of chromosome rearrangement shown in each photo involving:

   (a) Chromosome 15: _____

   (b) Chromosome 1 and 10: _____

   (c) Chromosome 14 and 21: _____

ISBN: 978-1-98-856655-9

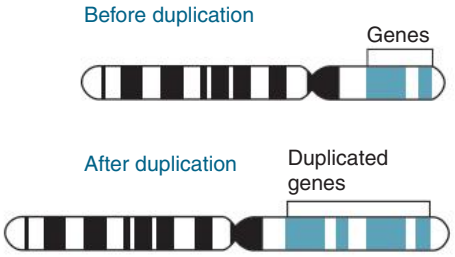
116 Gene Duplication

Key Question: How can mutations and preexisting variations help organisms adapt to their environments?

Recall that mutation is the source of all new alleles. Occasionally, whole genes, chromosomes, or genomes are duplicated. Gene duplication, if it does not produce serious side effects, allows natural selection to act on and change a gene while also maintaining an unchanged gene to perform the original role. There are many examples of this recorded in the genomes of organisms. Genes for the enzymes in snake venom can be traced to duplicated genes involved in producing enzymes in other parts of the body, including the salivary glands. Mutations can give populations the ability to evolve new features and abilities, allowing them to exploit new opportunities or changes in the environment.

Gene duplication allows the evolution of new abilities

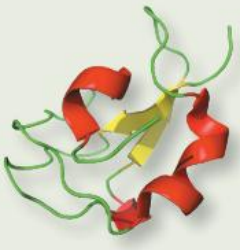
- Sometimes, having two genes with the same role (functionality) can be an advantage and both genes retain their original function. For example, when there is a high demand for a particular protein.
- Unless two copies of the same gene provide an advantage, one of the duplicated genes may develop a new function while the other copy continues on with its original function.
- Gene duplication is an important mechanism for generating new genetic material that can be subject to natural selection. Duplicated genes can then follow different evolutionary paths.
- Gene duplication is widespread. The nematode, *Caenorhabditis elegans*, has 49% genes duplicated, the fruit fly, *Drosophila melanogaster*, 41%, and yeast, *Saccharomyces cerevisiae*, 30%.



Gene duplication in Antarctic fish

Fish living in the near freezing waters of the Antarctic must have a way of ensuring their blood remains ice free. In many species, this is done by producing proteins with antifreeze properties. There are four major antifreeze proteins used by fish (called AFP types I - IV). The gene for the protein AFP III, found in Antarctic eelpout, is very similar to the gene that produces sialic acid synthase (SAS) (also found in humans).

Molecular studies have found that a slight modification to the SAS gene causes the production and secretion of AFP III protein. Importantly, the SAS gene product shows ice binding capability. It appears that duplication of the SAS gene produced a new gene that was selected for its ice binding capabilities and diverged to become the AFP III gene in Antarctic eelpout.



AFP III protein and SAS protein have similar structures and can be modified to have similar functions. This provides evidence for the likelihood of diversification of function after gene duplication.

Gene duplication in colobine monkeys

Gene duplication in colobine monkeys has enabled the production of enzymes that optimally perform similar functions in different bodily environments. Unlike most primates, the main food source of colobines is leaves. The leaves are fermented in the gut by bacteria. The monkeys, like ruminants, obtain their nitrogen through RNase-mediated digestion of RNA from these bacteria. In colobines, there are two forms of the RNase genes, **RNase1** and **RNase1B**, while other primates only have RNase1.

The optimal pH for the enzyme RNase1 and RNase1B are 7.4 and 6.3 respectively. In colobines, the pH of the digestive system is 6-7, but in other primates it is 7.4-8. RNase1B is six times more efficient at degrading RNA in the gut of colobines than RNase1. RNase1 is also expressed in cells outside the digestive system, where it degrades double stranded RNA and may defend against viral infection. RNase1B is 300 times less efficient at this function.


Red colobus

1. Explain how gene duplication can result in the evolution of a new gene function: _____

_____

_____

2. Describe the evidence that the AFPIII protein in the Antarctic eel pout may have evolved through a duplication of the SAS gene:

_____

_____

_____

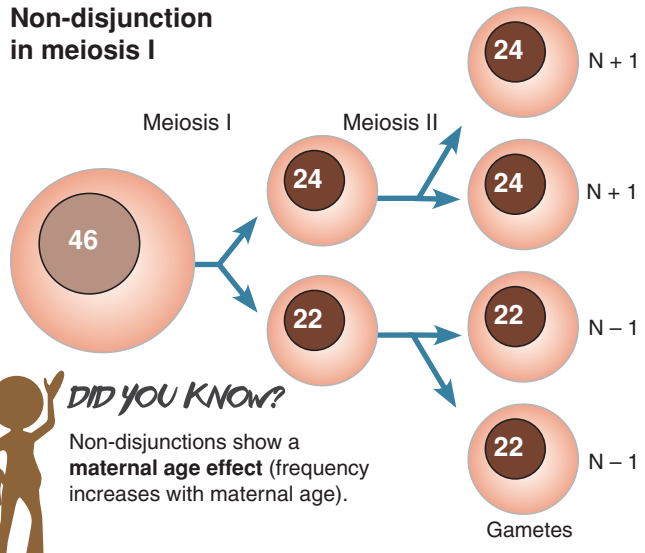
_____

ISBN: 978-1-98-856655-9

117 Non-disjunction can Produce Aneuploidies

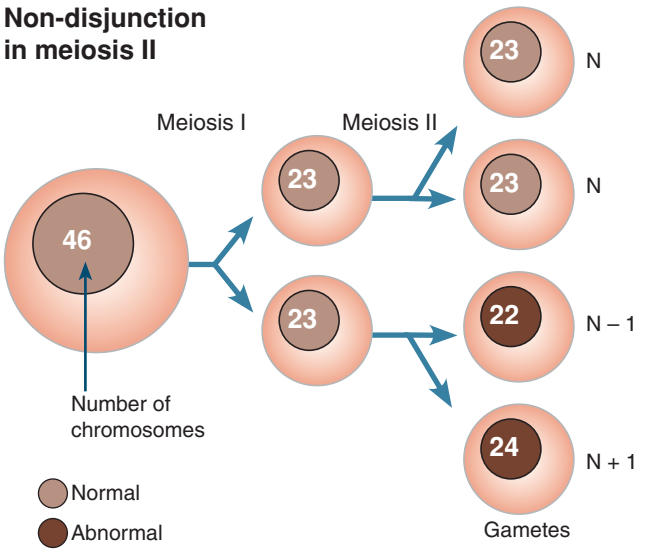
Key Idea: Non-disjunction during meiosis results in incorrect apportioning of chromosomes to the gametes.

In meiosis, chromosomes are usually distributed to daughter cells without error. Occasionally, homologous chromosomes fail to separate properly in meiosis I, or sister chromatids fail to separate in meiosis II. In these cases, one gamete receives two of the same type of chromosome and the other gamete receives no copy. This error is known as **non-disjunction** and it results in abnormal numbers of chromosomes in the gametes. Having an abnormal number of chromosomes in a cell is known as **aneuploidy**. The best known human aneuploidy is Down syndrome.

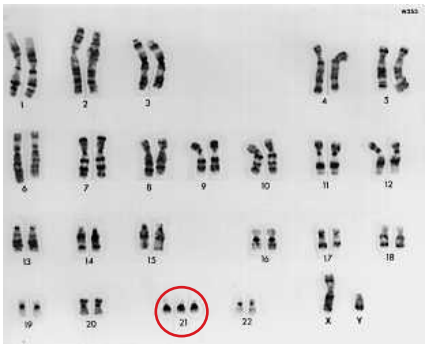


DID YOU KNOW?

Non-disjunctions show a **maternal age effect** (frequency increases with maternal age).



Down syndrome (trisomy 21)



Cytogenetics Department, Waikato Hospital

Down syndrome is the most common of the human aneuploidies. The incidence rate in humans is about 1 in 800 births for women aged 30 to 31 years, with a **maternal age effect** (the rate increases rapidly with maternal age). Around 95% of all cases result from **non-disjunction** of chromosome 21 during **meiosis**. When this happens, a gamete, most commonly the oocyte, ends up with 24 rather than 23 chromosomes, and fertilisation produces a trisomic offspring. *Above: A karyogram for an individual with trisomy 21. The affected chromosomes are circled.*

Datura stramonium

Agnieszka Kwiecie CC-BY 3.0

The plant *Datura stramonium* has 12 sets of chromosomes. There are 12 known aneuploids, each trisomic for a different chromosome. Each aneuploid has its own variety of seed pod shape, ranging from buckling (trisomy 3) to cocklebur (trisomy 6) and spinach (trisomy 10). All the aneuploids survive to be viable adult plants, indicating that plants are better able to accommodate genetic shuffling than animals.

1. Describe the consequences of non-disjunction during meiosis: _____

2. Explain why non-disjunction in meiosis I results in a higher proportion of faulty gametes than non-disjunction in meiosis II: _____

3. How does non-disjunction lead to aneuploidy? _____

ISBN: 978-1-98-856655-9

118 Aneuploidy in Sex Chromosomes

Key Idea: Nondisjunction of the sex chromosomes (X and Y) during meiosis may result in impaired development or spontaneous abortion of the fetus.

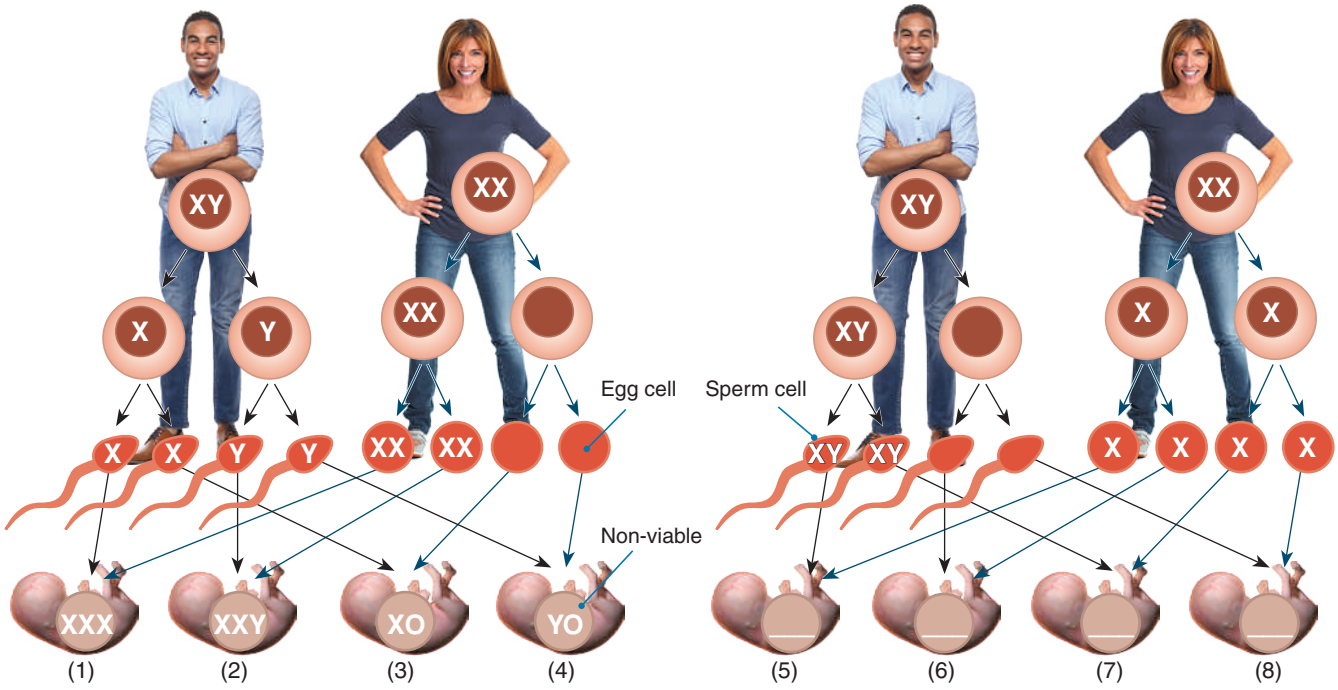
The X and Y chromosomes carry genes associated with sex, so the loss or gain of one of these chromosomes can have severe effects on sexual development, including low fertility. The Y chromosome carries the SRY gene, which triggers male development in the fetus. In females, only one X chromosome is ever fully active in a cell while the other is inactive, except for a few essential genes. The loss or gain of an X chromosome results in the under or over production of certain proteins, resulting in abnormal development.

Faulty egg production

Faulty meiosis during egg cell production can result in egg cells with 0 to up to 4 X chromosomes (nondisjunction in meiosis I and II).

Faulty sperm production

Faulty meiosis during sperm cell production can result in an uneven distribution of X and Y chromosomes.



Turner syndrome

Turner syndrome results from having only one sex chromosome (XO). The individual is female, with physical features that typically include a webbed neck and reduced stature. Although of average intelligence, individuals may have difficulty with spatial memory. The individual is usually infertile and will not develop secondary sexual characteristics without hormone treatment.

Klinefelter syndrome

Klinefelter syndrome results from at least one extra X chromosome (XXY, XXXY). Individuals are male and adults with Klinefelter syndrome are usually taller than average. Individuals are infertile and there may be a feminine distribution of fat tissue around the body. Intelligence varies from low to average.

1. Identify the sex chromosomes in each of the unlabelled embryos (above, right):

2. Identify which fetuses (1-8) are Turner or Klinefelter syndrome:

   (a) Turner: _____ (b) Klinefelter: _____

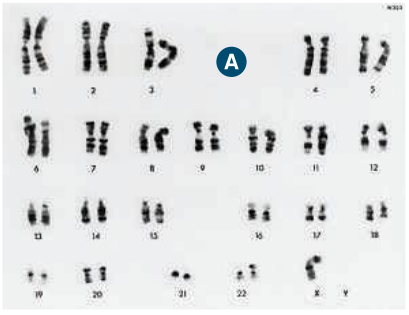
3. Why is the YO configuration (above) non-viable (i.e. there is no embryonic development)? _____

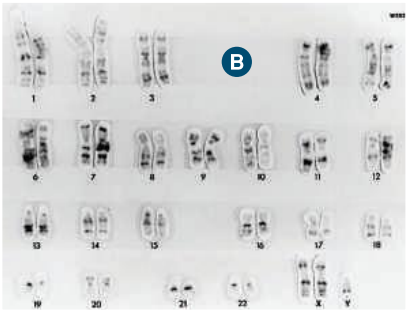
   _____

4. For the karyotypes **A** and **B**, below, circle the sex chromosomes and state:

   A: Chromosome configuration: _____ Sex of individual (M/F): _____ Syndrome: _____

   B: Chromosome configuration: _____ Sex of individual (M/F): _____ Syndrome: _____





ISBN: 978-1-98-856655-9

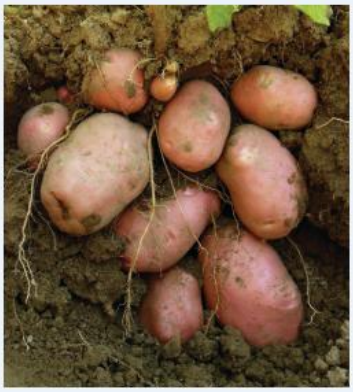
119 Polyploidy as a Source of Variation

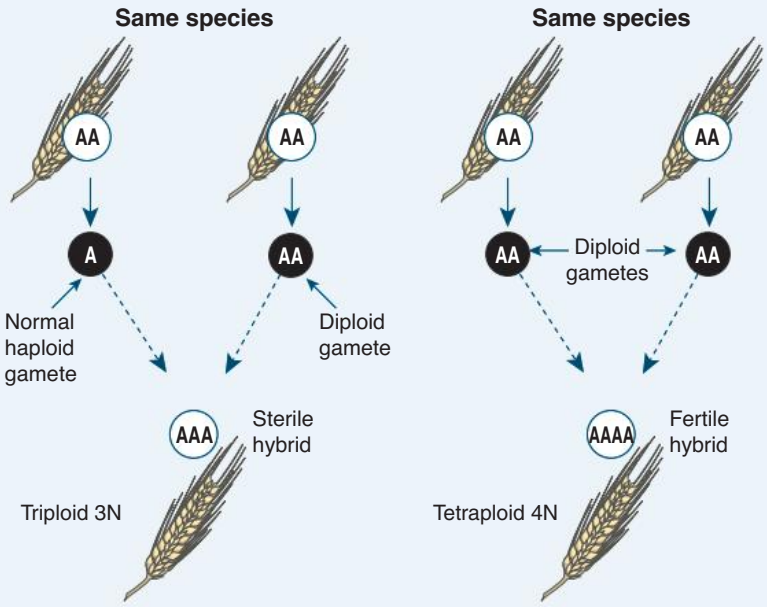
Key Idea: Polyploidy is a condition in which a cell or organism has three or more times the haploid chromosome number.

Polyploidy cells or organisms contain more than two haploid numbers of chromosomes (i.e. 3N or more). It arises when chromosomes fail to correctly separate during mitosis or meiosis (non-disjunction). Polyploidy is rare in animals, but common in plants, and has been important in the evolution and speciation of flowering plants (angiosperms). Polyploidy in plants produces a species that is reproductively isolated from the 'parent' species, and results in instant speciation. Allopolyploidy (involving different species) and autopolyploidy (involving the same species) are both recognised.

Autopolyploidy

Polyploids that arise within a species are called autopolyploids (the extra chromosomes come from another organism of the same species). Autopolyploidy occurs when chromosomes fail to separate during meiosis or when the cell fails to divide after the chromatids have separated. If a diploid gamete fuses with a haploid gamete, a triploid is formed. Triploids are generally unstable and sterile. However, if two diploid gametes fuse, the resulting tetraploid can be fertile.





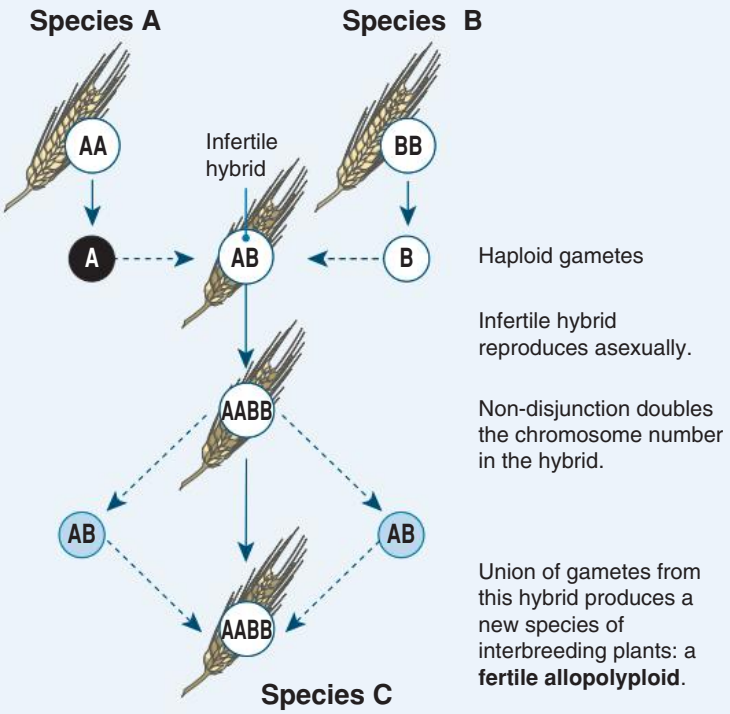
Potatoes (left) are autopolyploids. They have a number of ploidy levels, based on a haploid number of 12, ranging from diploid (2n=24) to hexaploid (6n=72). Cultivated potato varieties are tetraploid (4n=48).

Allopolyploidy

Allopolyploidy occurs when two species interbreed to produce a new hybrid with chromosomes from each of the parent species. The hybrid is infertile because the chromosomes cannot pair up. However, mitotic non-disjunction in the sterile hybrid can double the chromosome number and produce homologues, which can pair up during meiosis. Self-fertilisation may then produce a viable, fertile hybrid. Many commercial plant varieties are allopolyploids. They show greater heterozygosity and hybrid vigour than autopolyploids.

Honeyhuyue cc 3.0

Many crops are allopolyploids, including wheat, rice, and modern brassicas (above).



ISBN: 978-1-98-856655-9

Advantages of polyploidy

- The high frequency of polyploidy in plants indicates that polyploidy provides an adaptive advantage. Often this advantage is the result of hybrid vigour, where the hybrid shows improvements over the parents (e.g. by being larger or growing more vigorously). The increase in heterozygosity (heterozygous for a gene) reduces the frequency of (expressed) recessive mutations and also contributes to hybrid vigour.
- Polyploidy results in gene redundancy and provides opportunities to diversify gene function. Extra copies of the gene not required for its original function can be adapted for use in a different way. This can provide an evolutionary advantage. Many polyploids show novel variation or morphologies relative to their parental species.


Common wheat
6N = 42


Tobacco
4N = 48


Banana
3N = 27

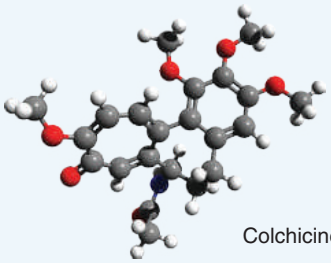

Boysenberry
7N = 49

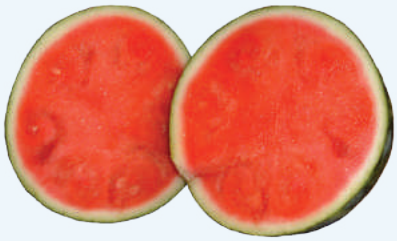

Strawberry
8N = 56


Kiwifruit
6N = 174

Polyploids can be induced

- New plant varieties can be made by inducing non-disjunction with chemicals. The induction of polyploidy is a common technique to overcome hybrid sterility during plant breeding.
- Chemicals such as colchicine (right) and N_2O gas inhibit spindle fibre formation and stop the separation of chromosomes during mitosis.
- In plants, seeds or seedlings are soaked in a solution of a spindle inhibiting chemical. The resulting plants will likely develop as polyploids. These can then be propagated and crossed (if fertile) to produce a new variety of plant.


Colchicine



- Seedless banana and watermelon fruits are produced on triploid plants, which cannot produce fertile gametes (therefore no seeds).
- Non-disjunction is induced in a diploid to produce a tetraploid, which is crossed with a normal diploid to produce the seedless triploid hybrid.

1. Explain how polyploidy can result in instant genetic isolation? _____

2. (a) What advantages do polyploid organisms often have over the parent species? _____

   (b) Explain the origin of these advantages: _____

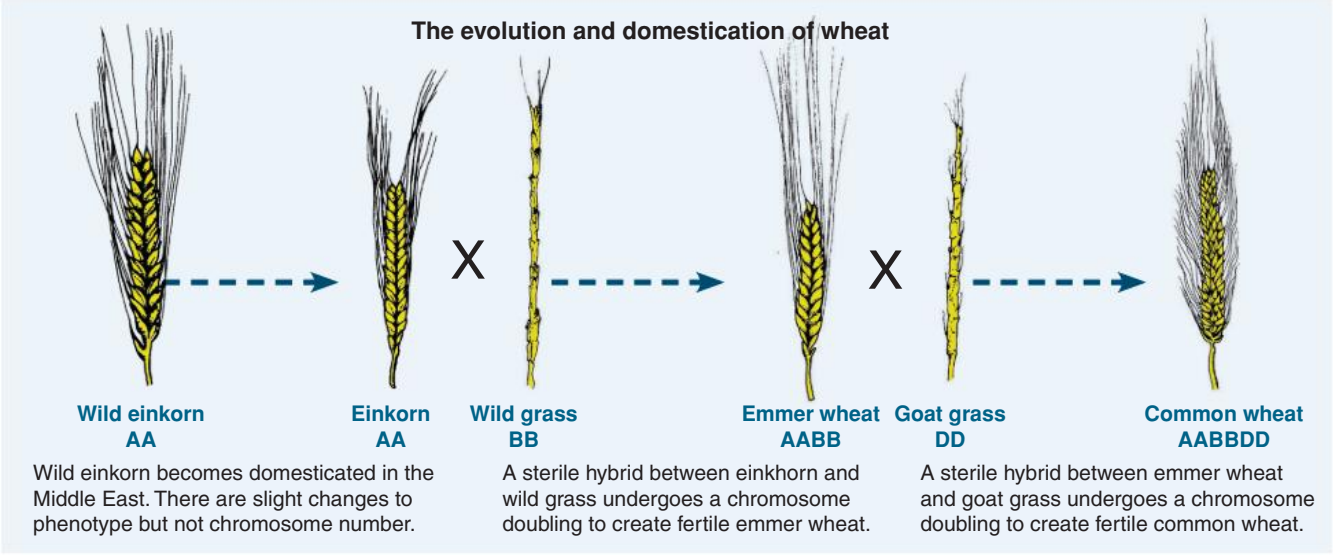
3. Distinguish between autopolyploidy and allopolyploidy: _____

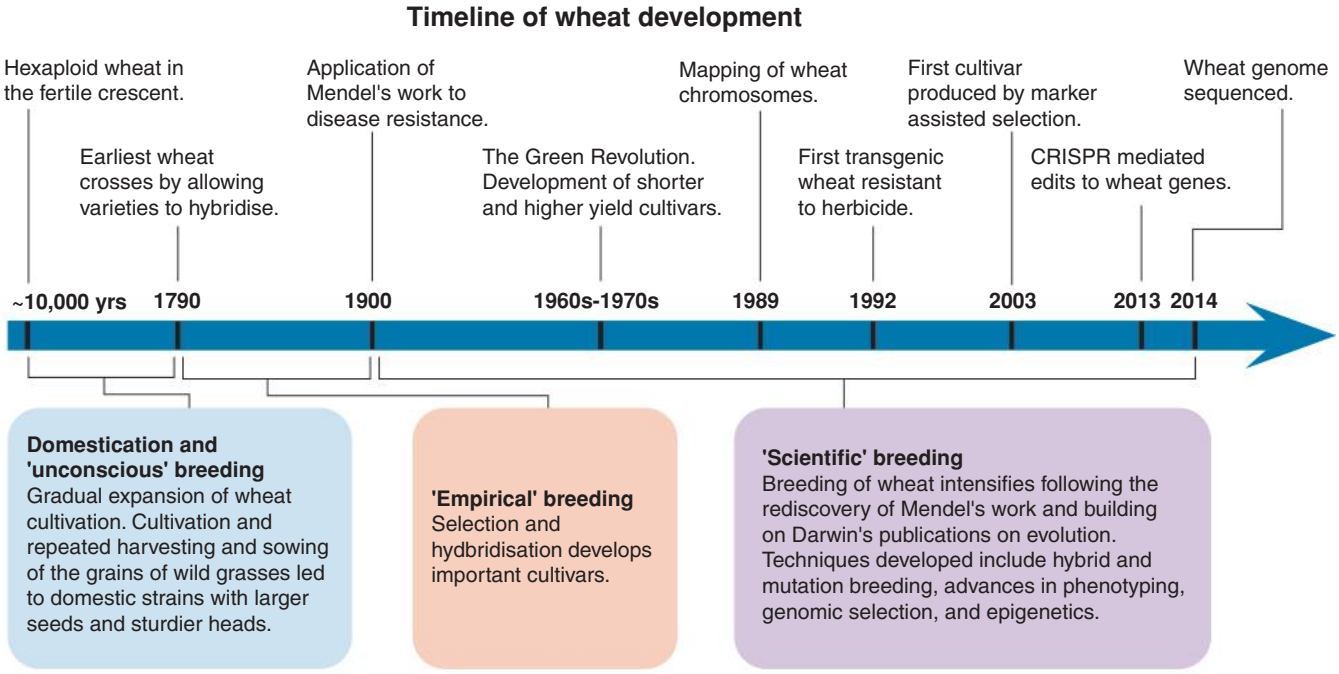
4. (a) How does non-disjunction result in polyploidy? _____

   (b) Using an example, explain how deliberate induction of non-disjunction can be used in producing crop varieties:

ISBN: 978-1-98-856655-9

Wheat has been cultivated for more than 9000 years and has undergone many genetic changes during its domestication. The evolution of modern bread wheat from its wild ancestors (below) involved two natural hybridisation events, accompanied by polyploidy (increasing number of complete sets of chromosomes). Once wheat became domesticated, selective breeding emphasised characteristics such as high protein (gluten) content, high yield, and pest and disease resistance. Hybrid vigour (improved characteristics) in wheat cultivars is produced by crossing inbred lines and selecting for desirable traits in the progeny (offspring). Increasingly, research is focused on enhancing the genetic diversity of wheat to provide for future crop development. With this in mind, there is renewed interest in some of the lower yielding, ancient wheat varieties, which possess alleles no longer present in modern inbred varieties.





5. List three phenotypic characteristics that would be desirable in a wheat plant: _____

_____

_____

6. How have both natural events and selective breeding contributed to the modern, high yielding wheat varieties?

_____

_____

_____

ISBN: 978-1-98-856655-9

120 Changes in a Population's Gene Pool

Key Idea: Natural selection and migration can alter the allele frequencies in gene pools.

The diagram below shows a hypothetical population of beetles undergoing changes as it is subjected to two 'events'. The three phases represent a progression in time, i.e. the same gene pool, undergoing change. The beetles have two phenotypes, dark and pale, determined by the amount of pigment deposited in the cuticle. The gene controlling this character is represented by two alleles **A** and **a**. Your task is to analyse the gene pool as it undergoes changes.

1. For each phase in the gene pool below fill in the following tables. The first has been done for you:

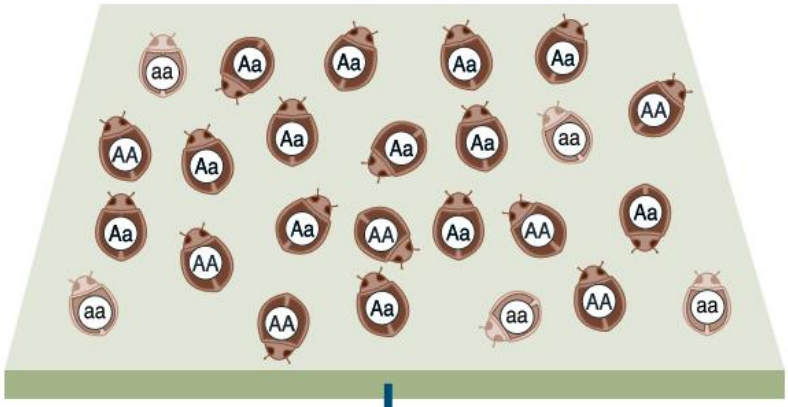
   (a) Count the number of 'A' and 'a' alleles separately. Enter the count into the top row of the table (left hand columns).
   (b) Count the number of each type of allele combination (AA, Aa and aa) in the gene pool. Enter the count into the top row of the table (right hand columns).
   (c) For each of the above, work out the frequencies as percentages (bottom row of table):

Allele frequency = No. counted alleles ÷ Total no. of alleles x 100

Phase 1: Initial gene pool

	A	a	AA	Aa	aa
No.	27		7		
%	54		28		

Allele types *Allele combinations*

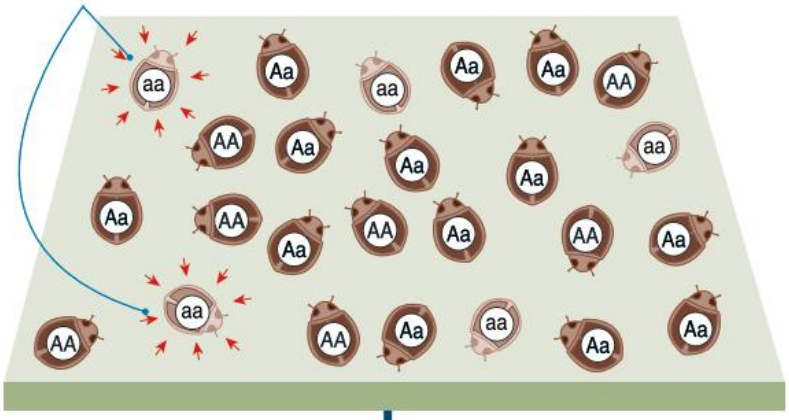


Two pale individuals died. Their alleles are removed from the gene pool.

Phase 2: Natural selection

In the same gene pool at a later time there was a change in the allele frequencies. This was due to the loss of certain allele combinations due to natural selection. Some of those with a genotype of aa were eliminated (poor fitness).
These individuals (surrounded by small red arrows) are not counted for allele frequencies; they are dead!

	A	a	AA	Aa	aa
No.					
%					



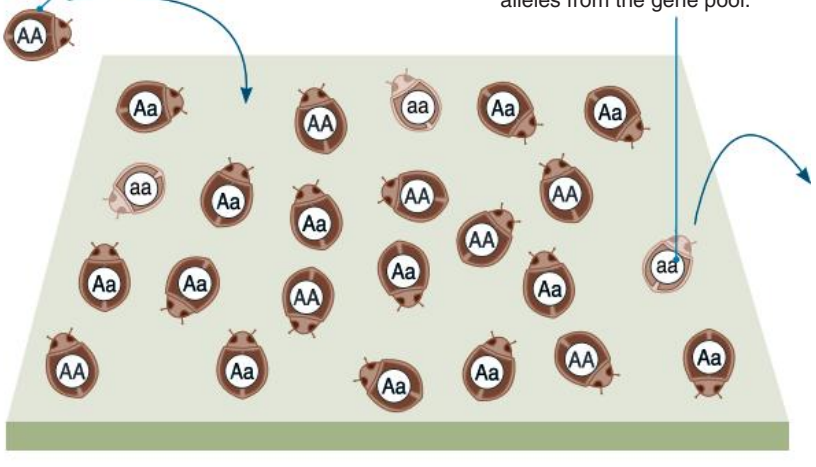
This individual is entering the population and will add its alleles to the gene pool.

This individual is leaving the population, removing its alleles from the gene pool.

Phase 3: Immigration and emigration

This particular kind of beetle exhibits wandering behaviour. The allele frequencies change again due to the introduction and departure of individual beetles, each carrying certain allele combinations.
Individuals coming into the gene pool (AA) are counted for allele frequencies, but those leaving (aa) are not.

	A	a	AA	Aa	aa
No.					
%					



ISBN: 978-1-98-856655-9

121 Selection Affects a Population's Gene Pool

Key Idea: The process of natural selection can be modelled using a simple series of steps on a spreadsheet.

Changes in gene pools are often modelled using physical representations of the genotypes in a population. This type of modelling is tedious and subject to human error. Modelling genotypic changes using a spreadsheet is quicker and allows the model to be changed to simulate different scenarios occurring in the gene pool.

Investigation 6.1 Investigating natural selection

Some natural selection labs work by manually placing tokens representing alleles into a bag and withdrawing them randomly to make genotypes. A certain genotype is then selected against by not returning it to the bag and the next generation is drawn from the remaining alleles. This exercise is long and difficult to manipulate, and it reduces the population numbers over time so that an accurate simulation is not entirely possible.

These problems can be solved by using a spreadsheet to compute allele changes over time. Once the formulae are in place, the spreadsheet can be manipulated in different ways to produce a more accurate, yet still simple, simulation. This spreadsheet can also be used to simulate genetic drift in Activity 122.

Download the spreadsheet from the BIOZONE Resource Hub or use the notes and screenshots below to recreate the spreadsheet yourself.

PART 1: Setting Up the spreadsheet

1. Open a new spreadsheet. The first thing to do is to switch off automatic calculation. This makes calculation of future allele frequencies simpler and under manual control, so that you can calculate them when you're ready. Each spreadsheet program will have slightly different ways of doing this. For Microsoft Excel click on the **Formulas** tab then on the **Calculation Options** menu and click **Manual**. Calculations can then be made using the **Calculate Now** button beside the Calculation Options menu or using the **F9** button.

2. The headings A and B represent the alleles A and a. This is necessary because the COUNTIF formula used later in the spreadsheet is not case sensitive (it does not recognise the difference between A and a).

3. 0.5 is the frequency of the A allele in the initial population (generation 0). The frequency of the B allele in the population is equal to 1-A. In our initial population, 50% of the alleles will be A and 50% will be B (A and a in the population).

	A	B	C	D
1	Allele frequency			
2	A	B		
3	0.5	=1-A3		
4				
5				
6	Gamete 1	Gamete 2		Zygote
7	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A7,B7)
8	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A8,B8)
9	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A9,B9)
10	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A10,B10)
11	=IF(RAND()<=$A$3,"A","B")	=IF(RAND()<=$A$3,"A","B")		=CONCATENATE(A11,B11)

4. The RAND formula produces a random number between 0 and 1 and compares it to the number in cell A3. If the random number is less than or equal to the number in cell A3 then an A is displayed in the cell. If the random number is greater, a B is displayed. The $ symbol tells the spreadsheet that cell A3 is a reference cell and must not change.

5. The CONCATENATE formula takes gametes A and B and puts them together to make the zygote.

6. Highlight cells and copy down all formulas to row 56 to produce 100 random gametes containing alleles A or B and 100 zygotes.

7. You now need to count up the number of AA, AB, and BB genotypes

	F	G	H	I	J
1	AA	AB	BB		Total Genotypes
2	=COUNTIF($D$7:$D$56,"AA")	=COUNTIF($D$7:$D$56,"AB")+COUNTIF($D$7:$D$56,"BA")	=COUNTIF($D$7:$D$56,"BB")		=SUM(F2:H2)
3					
4					

8. The COUNTIF formula counts up the number of AA, AB, and BB genotypes.

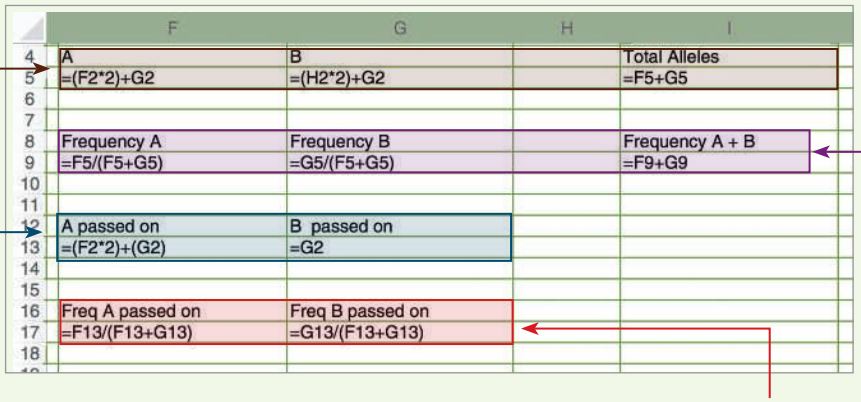
9. The SUM formula adds up the number of genotypes. It should add up to 50.

ISBN: 978-1-98-856655-9

10. Now you must calculate the number of A and B alleles present in this generation (Generation 1).In cell **F4** type the heading **A** and in cell **G4** type the heading **B**. In cell **I4**, type the heading **Total Alleles**.

11. Cell F5 adds up all the alleles from the AA genotype and the A alleles from the AB genotype. Cell **G5** adds up all the B alleles. Cell I5 adds up all the alleles. Click **Calculate Now** and you should see the number 100 appear.

12. Cells F9 and G9 calculate the frequency of As and Bs in Generation 1. Cell I9 adds up cell F9 and G9. This should add to 1.



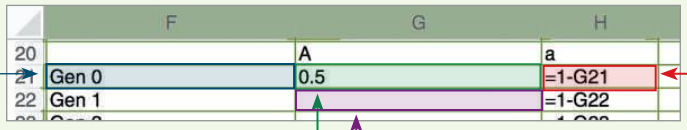
	F	G	H	I
4	A	B		Total Alleles
5	=(F2*2)+G2	=(H2*2)+G2		=F5+G5
6				
7				
8	Frequency A	Frequency B		Frequency A + B
9	=F5/(F5+G5)	=G5/(F5+G5)		=F9+G9
10				
11				
12	A passed on	B passed on		
13	=(F2*2)+(G2)	=G2		
14				
15				
16	Freq A passed on	Freq B passed on		
17	=F13/(F13+G13)	=G13/(F13+G13)		
18				

13. The **selection pressure** is against the recessive phenotype. The alleles in the recessive genotype (BB) will not be passed on, so the frequency of alleles in the population is different to the frequency of alleles that will be passed on. These cells calculate the **number** of alleles that will be passed on (excluding those in genotype BB).

14. These cells calculate the frequency of alleles that will be available to the next generation.

15. Finally, you must keep a record of each generation's allele frequencies before mating (i.e. before BB is excluded).

16. Gen 0 was your starting population. Copy down to cell **F31** to get ten generations.



	F	G	H
20		A	a
21	Gen 0	0.5	=1-G21
22	Gen 1		=1-G22

18. The frequency of a is simply 1–A. Copy down to **H31**.

17. The frequency of A in Gen 0 was 0.5

19. Click **Calculate Now**. Note the numbers that appear in cells **F9** and **G9**. Type the number in **F9** into **G22**. This is the frequency of the A allele in the first generation.

20. Now type the number in cell **F17** into cell **A3** and click **Calculate Now** to produce the second generation of alleles in cells F9 and G9. Again, enter the number in **F9** into **G23** and the number in **F17** into **A3** before clicking **Calculate Now**.

21. Each time you do this, the spreadsheet calculates a new generation of genotypes and their alleles based in the number you enter into A3.

22. Save your spreadsheet.

PART 2: Natural Selection lab

Now that you have built the spreadsheet and are familiar with it, you can begin the natural selection lab.

1. To do this you will select against the recessive phenotype (and hence the aa genotype, represented as BB in the spreadsheet). In this scenario any BB individuals never get to breed (it is irrelevant what the phenotype is, what is important is that no BB individuals will enter their alleles into the next generation).

2. To start the lab, make sure **0.5** is entered into cell **A3**. Enter **0.5** into cell **G21** and make sure the cells below them are clear. Highlight cells **F17** and **G17** and under the **Format** menu click **Cells**, then click the **Number** category and set it to **2** decimal places. Click **OK**.

3. It is also worth tracking the numbers of AA, Aa and aa individuals before breeding. You can do this by simply recording the numbers on a new part of the spreadsheet, the same way as recording the A and a allele frequencies. Theoretically, Generation 0 will start as 12.5 AA, 25 Aa, and 12.5 aa, but because only whole numbers of individuals are allowed these will need to be rounded to the nearest whole number that still

ISBN: 978-1-98-856655-9

produces a total of 50 (12, 26, 12). In cell **F35**, type the heading **Gen0**. **Highlight** the cell and copy it down to cell **F45**. In cell **G34**, type the heading **AA**, Cell **H34** type Aa and in **I34** type **aa**. Into cell **G35** type **12**, in **H35** type **26**, and in **I35** type **12**.

	F	G	H	I
35	Gen 0	12	26	12
36	Gen 1			
37	Gen 2			

4. Click **Calculate Now**.
5. Enter the results in cell **F9** into Generation 1 A (cell **G22**). Enter the numbers in **F2**, **G3,** and **H2** into **G36, H36**, and **I36**. Enter the number from **F17** into **A3** and click **Calculate Now** again.
6. Repeat this until you have ten generations of alleles.

PART 3: Graphing the data

1. You can now produce a graph of the results. Highlight the cells **F20** to **H31** and click **Insert** then click on a **line graph** with markers.
2. The graph should automatically produce two lines for A and a. Give the graph appropriate titles and axes labels by clicking **Add Chart Element** (depending on your spreadsheet programme) and selecting **title** and **axes** labels.
3. Repeat this for the AA, Aa, and aa individuals.
4. Print the graphs and staple them to this page.

1. (a) What happens to the frequency of the a alleles over ten generations when the aa genotype is totally excluded from passing its alleles to the next generation?

(b) What happens to the frequency of the A alleles over ten generations when the aa genotype is totally excluded from passing its alleles to the next generation?

(c) Why do your observations from (a) and (b) happen? _____

(d) What is the effect on the phenotypes over time? (Assume AA and Aa produce the same dominant phenotype and aa is the recessive phenotype).

(e) Predict what might happen if some of the aa genotype were able to breed: _____

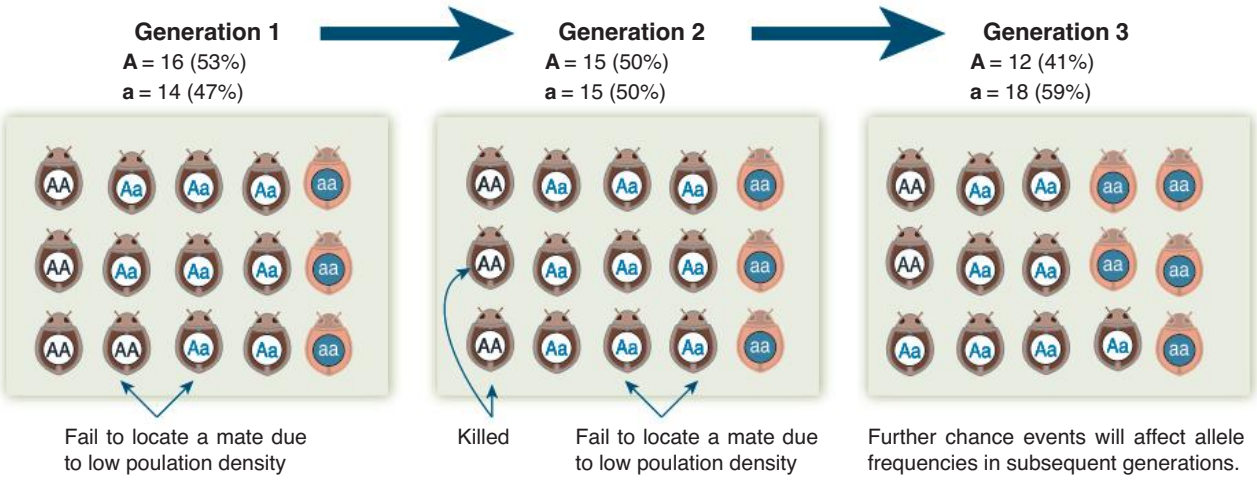
ISBN: 978-1-98-856655-9

122 Genetic Drift

Key Idea: Genetic drift describes the random changes in allele frequency that occur in all populations. It has a more pronounced effect in small populations.

Not all individuals, for various reasons, will be able to contribute their genes to the next generation. This random change in allele frequencies is called **genetic drift**. It is the result of 'sampling error' in the selection of alleles from the current gene pool for the next generation (not an error of how we might observe or record breeding events). The effect of genetic drift on a gene pool is more pronounced in small populations. Alleles may become **lost** from the gene pool (frequency = 0%) or **fixed** as the only allele present for the gene (frequency = 100%). Recall that in your natural selection spreadsheet, even starting with 0.5 *A* and 0.5 *a* the next generation of alleles were not 0.5 *A* and 0.5 *a* (even before selection had occurred). Due to the random mating set in the simulation, sometimes more or less than 0.5 *A* alleles were passed on. This directly affects the frequencies in the next generations's gene pool. Even without any selection, random changes in the gene pool can have important effects.

The genetic makeup (allele frequencies) of the population changes randomly over a period of time

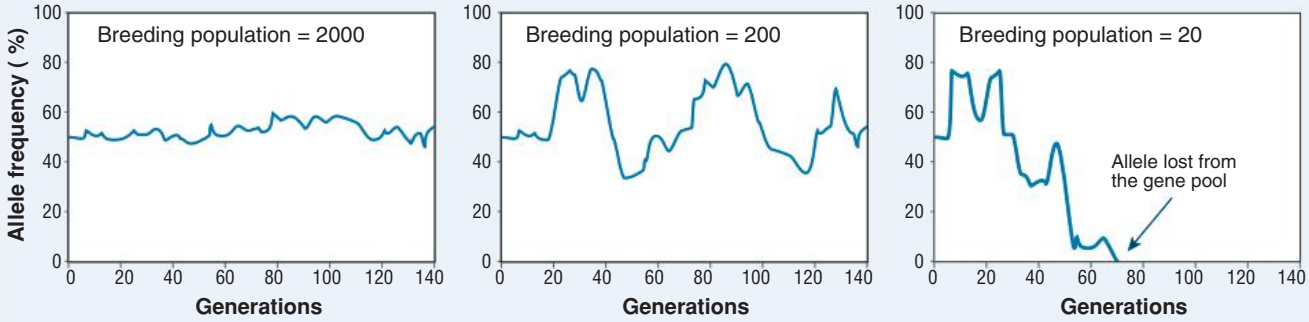


This diagram shows the gene pool of a hypothetical small population over three generations. For various reasons, not all individuals contribute alleles to the next generation. With the random loss of the alleles carried by these individuals, the allele frequency changes from one generation to the next. The change in frequency is directionless as there is no selecting force. The allele combinations for each successive generation are determined by how many alleles of each type are passed on from the preceding one.

Computer simulations of genetic drift

- Computer simulations are used to carry out population experiments which are impractical to fully observe in wild populations. For instance, the generation times may be too long or obtaining genetic samples impossible.

Below are displayed the change in allele frequencies in a computer simulation showing random genetic drift. The breeding population progressively gets smaller from left to right. Each simulation was run for 140 generations.



Large breeding population
Fluctuations are minimal because large numbers buffer the population against random loss of alleles. On average, losses for each allele type will be similar in frequency and little change occurs.

Small breeding population
Fluctuations are more severe in smaller breeding populations because random changes in a few alleles cause a greater percentage change in allele frequencies.

Very small breeding population
Fluctuations in very small breeding populations are so extreme that the allele can become fixed (frequency of 100%) or lost from the gene pool altogether (frequency of 0%).

- You can use the spreadsheet you made in Activity 121 to investigate the effect of population size on allele frequency changes due to genetic drift (see next page). You will need to modify the spreadsheet to make random changes to the frequency with which alleles are passed on to the next generation. This simulates random events, removing alleles from the gene pool (as above).

ISBN: 978-1-98-856655-9

Investigation 6.2 Modelling genetic drift

Part 1

1. Modify the spreadsheet you made for Activity 121 to complete this simulation. Save the spreadsheet under a new name.
2. Open the spreadsheet. In cell **F13** (under the heading A passed on) change the formula to **=(F2*2*RAND())+(G2*RAND())**. This randomly selects a number of A alleles from the total available to pass to the next generation.
3. In cell **G13**, change the formula to **=(H2*2*RAND())+(G2*RAND())**. This randomly selects B alleles.

F	G
AA	AB
=COUNTIF($D$7:$D$56,"AA")	=COUNTIF($D$7:$D$56,"AB")+COUNTIF($D$7:$D$56,"BA")
A	B
=(F2*2)+G2	=(H2*2)+G2
Frequency A	Frequency B
=F5/(F5+G5)	=G5/(F5+G5)
A passed on	B passed on
=(F2*2*RAND())+(G2*RAND())	=(H2*2*RAND())+(G2*RAND())
Freq A passed on	Freq A passed on
=F13/(F13+G13)	=G13/(F13+G13)

4. Set cell **A3** to **0.5** and click **Calculate Now**. Record the A and a frequency for each generation, the numbers of AA, Aa, and aa individuals and produce graphs for twenty generations (stop if an allele becomes fixed) as in part 2 of Activity 102. Note Gen 0 AA = 13, Aa = 26 and aa = 13.

Part 2

1. You can now change the population number and see how genetic drift affects allele frequencies. In cell **F2**, change the COUNTIF formula to **=COUNTIF($D$7:$D$26,"AA")** to count only the first 20 individuals.
2. In cell **G2**, change the formula to **=COUNTIF($D$7:$D$26,"AB")+COUNTIF($D$7:$D$26,"BA")** and in cell **H2** change the formula to **=COUNTIF($D$7:$D$26,"BB")**. Click **Calculate Now** and cell J2 should show 20.
3. Set cell A3 to **0.5** and click **Calculate Now**. Record the 'A' and 'a' frequency for each generation, the numbers of AA, Aa, and aa individuals and produce graphs for twenty generations (stop if an allele becomes fixed). Note Gen 0 AA = 5, Aa = 10 and aa = 5.
4. Now repeat the above but set the COUNTIF formulae to count to row **106**. Highlight cells **A56** to **D56** and copy them down to row **106**. Click **Calculate Now** and cell J2 should show 100.
5. Set cell **A3** to **0.5** and click **Calculate Now**. Record the A and a frequency for each generation, the numbers of AA, Aa, and aa individuals and produce graphs for twenty generations (stop if an allele becomes fixed) Note Gen 0 AA = 25, Aa = 50 and aa = 25.

1. What is genetic drift, and why are its effects more pronounced in smaller populations?_____

2. Which of the simulations you did above appears to have the most fluctuations in the A and a alleles? Explain why:

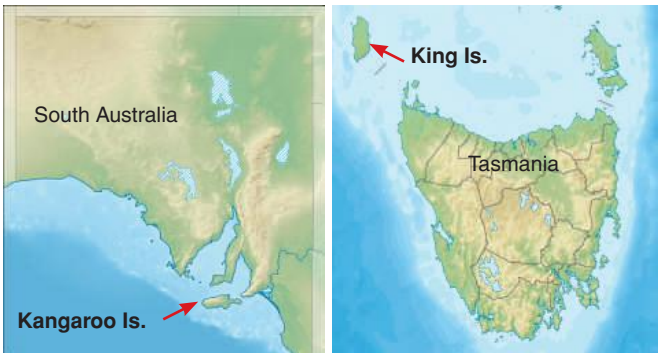
3. Suggest why genetic drift is regarded as an important process in the evolution of small populations. You can use your simulation as evidence if you wish:

ISBN: 978-1-98-856655-9

Island platypus and the perils of genetic drift

- A 2012 study of genetic diversity in platypus populations on mainland Australia, Tasmania, King Island, and Kangaroo Island has revealed very low immunological diversity in the island populations. The study (Lillie *et al.*) looked at the diversity of the MHC DZB gene and three MHC associated markers, all of which are involved in immune function. High allelic diversity in immune genes is important because it provides the variation necessary to resist different kinds of diseases. Without genetic variation, the population is likely to have low resistance to new diseases and environmental change.
- The study found that populations on the Australian mainland and in Tasmania have high levels of genetic diversity within their populations, with 57 DZB alleles identified in 70 individuals. However platypuses on King Island and Kangaroo Island (see maps), had very low levels of genetic diversity. For the King Island populations, there was no variation at all (only one allele at the DZB locus).
- Why is the genetic diversity of these island populations so low compared to the mainland populations? The Kangaroo Island population was founded from an introduction of around 20 animals in the 1930s and 1940s (founder population). The population on King Island is endemic, separated for some 14,000 years since the last ice age.
- Inbreeding in a small population and genetic drift have resulted in the loss of alleles and a dangerously low diversity in immune genes. These island populations will now need careful management to protect them from disease risk.

Klaus cc 2.0



Tentotwo cc 3.0

Nzeemin cc 3.0

Source: Diversity at the Major Histocompatibility Complex Class II in he Platypus, *Ornithorhynchus anatinus* Mette Lillie *et. al.* Journal of Heredity 2012:103(4):467–478

4. What factors have contributed to the low genetic diversity of the platypus populations on King Island and Kangaroo Island? Explain their effect(s):

5. Why would genetic drift have more impact on the genetic diversity of these populations than those on the mainland?

6. (a) Describe why a population with low MHC diversity is more likely to be affected by a new disease than a population with high MHC diversity:

   (b) Why is it important to try to keep small, isolated populations free of new diseases?

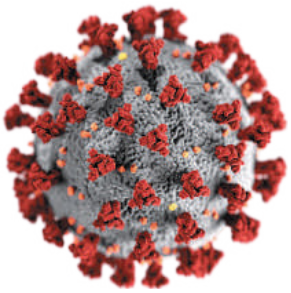
ISBN: 978-1-98-856655-9

123 Mutations and Gene Pools

Key Idea: The gene pool of a population can be changed by mutations. If a mutation gives a selective advantage, it will persist in a population.

A population's gene pool can be thought of as a collection of all its alleles. Mutations can create new alleles, resulting in changes to the gene pool over time and this gradual alteration of the gene pool is what we call evolution. Some mutations are beneficial and will be selected for, over time. Other, harmful mutations may die out. If the rate of mutation in a population is high, many new alleles will be passed to the offspring and into the gene pool. If the rate of mutation is low, the genetic variation in the gene pool will be stable or decrease. Mutations in isolated populations can be more prevalent than in the parent population.

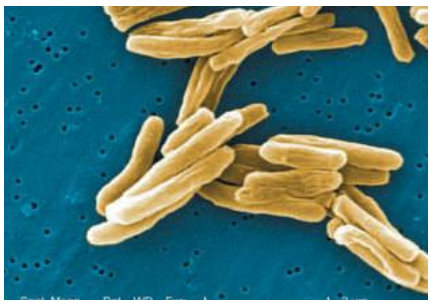
Mutation in SARS-CoV-2 virus



CDC

The SARS-CoV-2 virus, responsible for the Covid-19 pandemic, has undergone rapid mutation since it emerged in early 2020. Mutations have resulted in waves of human infection by different variants and some, such as Delta and Omicron, are extremely transmissible. It is likely that further mutations will cause new variants to emerge as the pandemic continues.

Antimicrobial resistance



CDC

Multi drug-resistant *Mycobacterium tuberculosis* causes a form of tuberculosis which is not affected by the most common drugs used to treat the disease. As bacteria multiply and mutate, drug resistant forms become more common in the population. These favourable genes are passed to offspring so that the drug resistant forms become more prevalent.

Albinism

Albinism is a mutation that affects pigmentation of the skin, fur, eyes, feathers and scales. One of the mutations leading to albinism prevents formation of tyrosinase, which ultimately leads to production of melanin. A population of albino wallabies has grown on an offshore island in Tasmania, Bruny Island, where lack of predation has allowed numbers to multiply.

Increases in harmful mutations

Artificially selecting for specific traits when breeding dogs has led to an accumulation of harmful mutations in certain breeds. This has led to specific, disease causing mutations becoming more common. High levels of inbreeding can lead to high levels of harmful traits, including a high incidence of deafness in dalmatians, and hip dysplasia in German Shepherds.

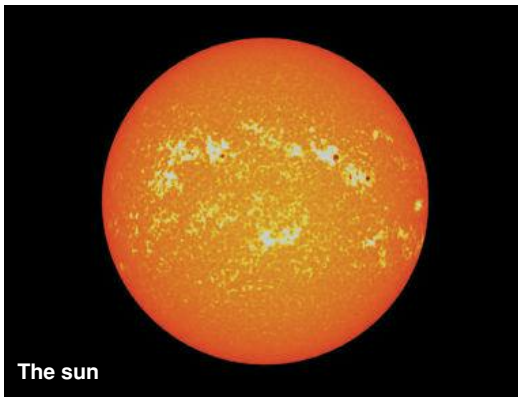
1. Why is a large gene pool beneficial to the survival of a species?_____

2. Why are very few albino animals found in wild populations?

3. A genetic bottleneck is caused by a large reduction in the gene pool of a species either because of a natural event such as a bush-fire, or by humans artificially selecting for specific traits in domesticated species. Explain why bottlenecks are harmful to the survival of species:

ISBN: 978-1-98-856655-9

124 Chapter Review: Did You Get It?


The sun

1. (a) Explain why the ultra-violet radiation from the sun is dangerous:

(b) How do certain viruses induce mutations in cells?

2. What is the difference between exons and introns?

3. How could a mutant allele in a population of organisms on an offshore island become the most common allele?

4. A large protein, titin, is coded for by the TTN gene. This protein acts as a spring inside the heart muscle, and defects can cause the left ventricle to enlarge and become weak. Different mutations are known to affect the TTN gene, including frameshift, nonsense, and missense. Explain how each of these mutations could affect the titin protein:

5. An original DNA sequence is as follows: **GCG TGA TTT GTA GGC GCT CTG**

   For each of the following DNA mutations, state the type of mutation that has occurred:

   (a) **GCG TGT TTG TAG GCG CTC TG**

   (b) **GCG TGA TTT GTA AGG CGC TCT G**

   (c) **GCG TGA TTT GGA GGC GCT CTG**

   (d) **GCG TGA GTA GGC GCT CTG**

6. For the DNA sequence **G** G T C T C C G T A A T A T T show the effect of the following by writing the new DNA sequence (note: position one in the sequence starts with the bold **G** on the left hand side):

   (a) A deletion of the T at position 5:

   (b) A substitution of C at position 7 with A:

   (c) A deletion of the bases from positions 10 - 12:

7. What is meant by the term, 'code degeneracy'?

8. Explain how a mutation might cause a new phenotype:

ISBN: 978-1-98-856655-9

CHAPTER 7

Biotechnology

Key terms

biodiversity
CRISPR
gene drives
gene editing
genetically modified organisms (GMOs)
genetic screening
nanomedicine
reproductive technologies
transgenes

Activity number

Inquiry question: *How do genetic techniques affect Earth's biodiversity?*

Uses of biotechnology

Key skills and knowledge

Benefits of genetic technology

Key skills and knowledge

Genetic technology and Earth's biodiversity

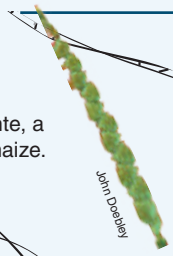
Key skills and knowledge

125 Biotechnology in the Past, Present, and Future

Key Idea: Humans have been manipulating the transfer of genetic information between organisms ever since plants and animals were first domesticated, over 10,000 years ago. Selective breeding, i.e. the breeding together of organisms with desirable traits, was the earliest form of genetic manipulation. In the last few decades, humans have developed sophisticated methods to manipulate genetic information to produce organisms with beneficial traits, or reduce the occurrence or impact of disease. For example, the insertion of a gene from one species into another completely different species is now common-place. The timeline below outlines significant steps in the history of human genetic manipulation.

~8,000 BC

Cultivation of grains and domestication of animals.



Teosinte, a wild maize.

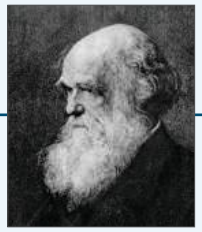
John Doebley

~3,000 BC

Mules created by crossing donkeys and horses.

3,000 BC-AD 1800

Breeds of plants and animals refined. Many of today's common varieties and breeds appeared by the mid 1800s.



AD 1850

1859 Charles Darwin publishes Origin of Species in which he details the selective breeding of pigeons to produce breeds with radically different appearances.

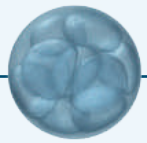
1890 Rabbits are born from embryos that were implanted in surrogate mothers.

AD 1920

1926 Hybridisation of plant crops produces higher yielding seeds.

1930 First collection of bovine embryos for transfer to surrogate mothers.

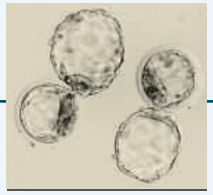
1940 Mutagens used on plants to alter genes and produce new plant varieties.



AD 1950

1951 First successful transfer of bovine embryos to surrogate mothers.

1953 Structure of DNA proposed by Watson and Crick.



Cloned embryos

AD 1970

1972 First *in vitro* recombinant DNA. Frozen mouse embryo revived, implanted, and born.
1973 First transgenic bacteria produced.

1976 Prenatal genetic diagnosis using DNA.
1978 First human baby from *in vitro* fertilisation born.
1979 Embryo splitting to produce twin lambs. Insulin produced by GM bacteria.

PCR machine

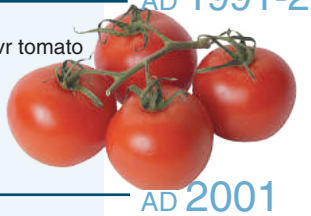
AD 1980-1990

Achievements include: *In vitro* fertilisation of bovine ovum, transgenic mice, PCR, transgenic pigs, first transgenic plant resistant to insects, location of gene for Huntington's disease, transgenic maize, transgenic plants for pharmaceuticals.

AD 1991-2000

Achievements include: gene therapy, plant IVF, Flavr savr GE tomato commercially available, cloning produces Dolly the sheep, completion of first draft of the human genome.

Flavr Savr tomato



www.glofish.com

AD 2001 onwards

Achievements include: first gene therapy successes (SCID), genetically modified rhesus monkey, mouse with two female parents born, various cloned animals produced, Glofish - the first genetically engineered pets commercially available, development of CRISPR gene editing technology.

ISBN: 978-1-98-856655-9

Applications of Genetically modified organisms (GMOs)

- Recall that organisms that have had their DNA altered are called genetically modified organisms (GMOs). Incorporation of a new gene into a species can optimise their performance (e.g. growth) or stimulate production of a desirable product (e.g. higher vitamin levels). Current applications are highly varied and include food and enzyme technology, industry and medicine, environmental cleanup, and agriculture. Some medical and agricultural applications are given below.

GMOs and agriculture

In Australia, the use of genetically modified crops is tightly regulated and approval is needed before they can be grown. Strict regulation is in place because transgenes (literally 'transferred genes') are easily spread between plant species and could result in plants accidently acquiring foreign genes.

What GM crops are grown in Australia?

- Three GM crops are currently grown in Australia: canola (right), cotton, and safflower. There are a number of experimental field trials also in progress. These include bananas, barley, ryegrass, sugarcane, and wheat.

Why GM crops?

- There are many different reasons to genetically modify crops. Common uses are to increase crop yield, decrease the cost of production, improve crop genetics, or to enhance nutritional qualities.

IRRI CC2.0

Scott Bauer, USDA ARS

Crop improvement: The nutrient content of crops can be enhanced to have higher protein or vitamin levels (e.g. golden rice has higher levels of β-carotene, which is needed to make vitamin A). Plants can also be engineered to use less water or to grow in conditions they could not normally tolerate (e.g. saline soils).

Pest or herbicide resistance: Large amounts of money are spent on spraying chemicals to control plant pests. Plants can be engineered to express genes for insect toxins or herbicide resistance. Pest resistant crops do not require spraying and herbicide resistance allows the grower to control weeds without damaging the crops.

Extending shelf life: Food that spoils before it can be sold reduces profit. Shelf life in fresh produce (e.g. tomatoes) can be extended by switching off the genes for specific enzymes involved in the fruit ripening process (e.g. the enzymes involved in softening of the fruit wall or controlling the production of ethylene).

GMOs and pharmaceuticals

Production of bioactive proteins: Transgenic bacteria are widely used to produce desirable commodities, such as hormones. Large quantities of a product can be produced commercially in large bioreactors. One example is injectable human insulin (above) produced in recombinant bacteria or yeast. Transgenic sheep carrying the human gene for a protein called α-1-antitrypsin, produce the protein in their milk. The antitrypsin is extracted from the sheep's milk and can be used to treat hereditary forms of the breathing disorder emphysema.

Vaccine development: Genes encoding antigenic components (e.g. viral proteins) are inserted into a bacterial cell, which then expresses the genes. The gene product is purified to make a vaccine and generates an immune response without the risk of ever causing the disease.

1. Choose one of the following topics to research:

   (a) Choose one genetically modified (GM) crop grown in Australia (canola, cotton, or safflower) and research the advantages and disadvantages of its use compared to a non-GM crop. Summarise your ideas on a shared document for the class to comment on.

   (b) Edible plants can be engineered to carry antigenic proteins. If you could save lives by producing an edible vaccine in a GM crop would you do it? Research this topic and write a discussion on the potential of edible vaccines to deliver vaccines safely and at low cost. Post your ideas in a shared document for your classmates to see and discuss.

ISBN: 978-1-98-856655-9

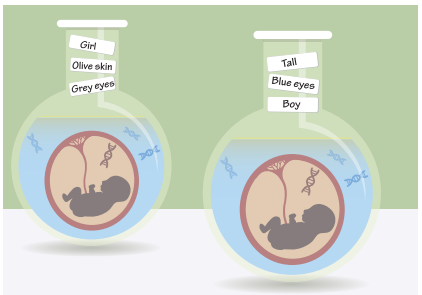
126 The Ethics of GMO Technology

Key Idea: The very nature of applying technology to biology raises ethical issues over just how far the technology should be taken, or how we are interfering with life.

Bioethical issues arise when any living organism is used by humans for any purpose. Bioethical issues are also generated when humans use technology to gain information about the biological nature of another human. An example might be the possibility of using someone's genomic information for a purpose different to its original intent (e.g. using it to disadvantage someone attempting to obtain health insurance). Other bioethical issues arise when genomic analysis indicates a heritable and life threatening disease. Should you be told, and would you or should you have to tell a partner with whom you might have children?

Genetic screening in Australia

- The genetic screening of gametes, embryos, children, and adults for some diseases is now possible. Genetic screening has many applications including in the detection and treatment of diseases.
- Genetic screening has many positive applications, but it raises a number of bioethical issues. This is particularly the case for the screening of embryos and fetuses because it may result in their destruction if they have genetic defects, or even an undesirable genotype (e.g. the wrong sex).



Diagnostic testing: A person may have symptoms typical of a particular genetic disorder. Genetic screening is used to determine if the person has the gene associated with a particular disease or not.

Pharmacogenetics: Genetic screening can be used to help decide what type, or dose, of medicine will be best for an individual. Targeted treatment can increase the chances of the medicine working.

USAF

Newborn screening: Newborns are screened for a range of metabolic disorders (e.g. phenylketonuria). If a disease is detected, treatment can begin immediately and the child's prognosis is improved.

Arguments for genetic screening

- Testing allows potential carriers to be screened for a disease so they can decide whether they have children or not. This is important for diseases that do not show any symptoms until later in life (e.g. Huntington's disease).
- Researchers can study individuals with the gene(s) associated with a disease and this may help them to develop a treatment or cure for that disease.
- Knowing a person's genetic make-up can be used to optimise drug therapies and improve treatment outcomes.
- Knowing the risk of developing a disease allows informed decisions to be made about medical options. For example, breast cancer can be treated, so an individual may decide to increase screening to increase the chance of early detection. They may choose to reduce risk factors (e.g. breast removal if they are at high risk of breast cancer).
- The discovery of a genetic defect in an unborn child provides an opportunity to come to terms with the situation and prepare for the delivery and ongoing care of a special needs child.

Arguments against genetic screening

- Genetic tests can only tell you if you carry a gene for an associated disorder. They cannot yet predict when and if you will develop the disease, or to what extent. Testing therefore carries the risk of causing unnecessary anxiety.
- An individual's privacy may be compromised by testing. The knowledge that you may develop a genetic disorder in the future could be used against you (e.g. medical insurance could be declined or an employer may no longer want to employ you)
- Designer babies could be produced where parents pick certain characteristics they want their child to have. This is already seen in countries where more value is placed on the birth of a boy child than a girl, and unwanted female fetuses are terminated.
- The discovery of a genetic defect in an unborn child may lead to the decision to terminate the pregnancy, an action some people believe is morally wrong because they feel it devalues human life.

1. Describe some of the benefits of genetic screening:_____

ISBN: 978-1-98-856655-9

Reproductive technologies

- Reproductive technologies have helped many people who are unable to conceive naturally to have children. However, these technologies also produce many bioethical issues including the ethics of cloning, sex or trait selection, and even issues surrounding conservation and the resurrection of extinct species.

Assisted reproduction

Assisted reproduction includes a wide array of reproductive technologies. It has been used for decades to help couples have children when they are unable to conceive naturally. Technologies include *in-vitro* fertilisation, in which sperm and an egg cell are mixed in the laboratory to produce an embryo. The embryo is then transplanted into the mother (or a surrogate). Issues arise around the selection of the embryos, age of parents, and cost to society.

Cloning

Embryo transferred Charolais calves with their Angus and Hereford recipient mothers.

Plants and animals can both be cloned. Plant cloning is widely used with few bioethical concerns, but animal cloning is less common and more ethically problematic because of welfare issues and the implications for humans. In theory, a cloning technique that successfully clones an animal could also be used on humans. Cloned human cells could produce immunocompatible organs for transplant. Ethical issues exist around cloning humans.

Conservation

Spiny daisy (*Acanthocladium dockeri*)

Reproductive technologies are often used in conservation. The Wollemia pine (*Wollemia nobilis*) and spiny daisy (above) are critically endangered species that have been saved from extinction by cloning from cuttings. Cloning and embryo transfer may be the only chance to save some animal species on the brink of extinction (e.g. Northern white rhino). Sometimes, reproductive technologies are used to reduce pest populations by sterilisation or contraception.

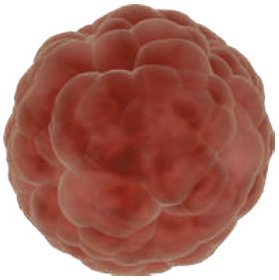
Ethical issues around reproductive technologies include:

Possible wrongs to the community by the use of IVF

- IVF is a costly procedure.
- Couples who can afford IVF may be putting money and effort into conception instead of the community.
- The community may have to bear the cost of IVF and welfare for financially struggling parents.
- Offspring with health issues due to IVF may be an ongoing burden to the community.

The rights of the pre-embryo (blastocyst)

- Multiple blastocysts are transferred to a woman's uterus to increase the chances of implantation. After implantation, many of these blastocysts are destroyed by selective pregnancy reduction.
- Many different ideas and definitions exist over the start of personhood or individuality. What are the ethics around destroying embryos?



Possible wrongs to those directly involved in an IVF conception

- Multiple blastocysts are transferred to a woman's uterus to increase the chances of successful implantation. This may result in a multiple pregnancy, which can have psychological and health effects on the parents.
- In cases where IVF requires a gamete donor (not from either of the couple), what are the rights of that donor?
- IVF is expensive and may create a financial strain on the couple.

Possible wrongs to the offspring by the use of IVF

- There is some evidence to suggest that IVF babies have a higher chance of some medical problems, including pre-term birth, low birth weight, spina bifida, and heart defects.
- Parents with genetic defects preventing them from conceiving naturally may pass these defects to the offspring.

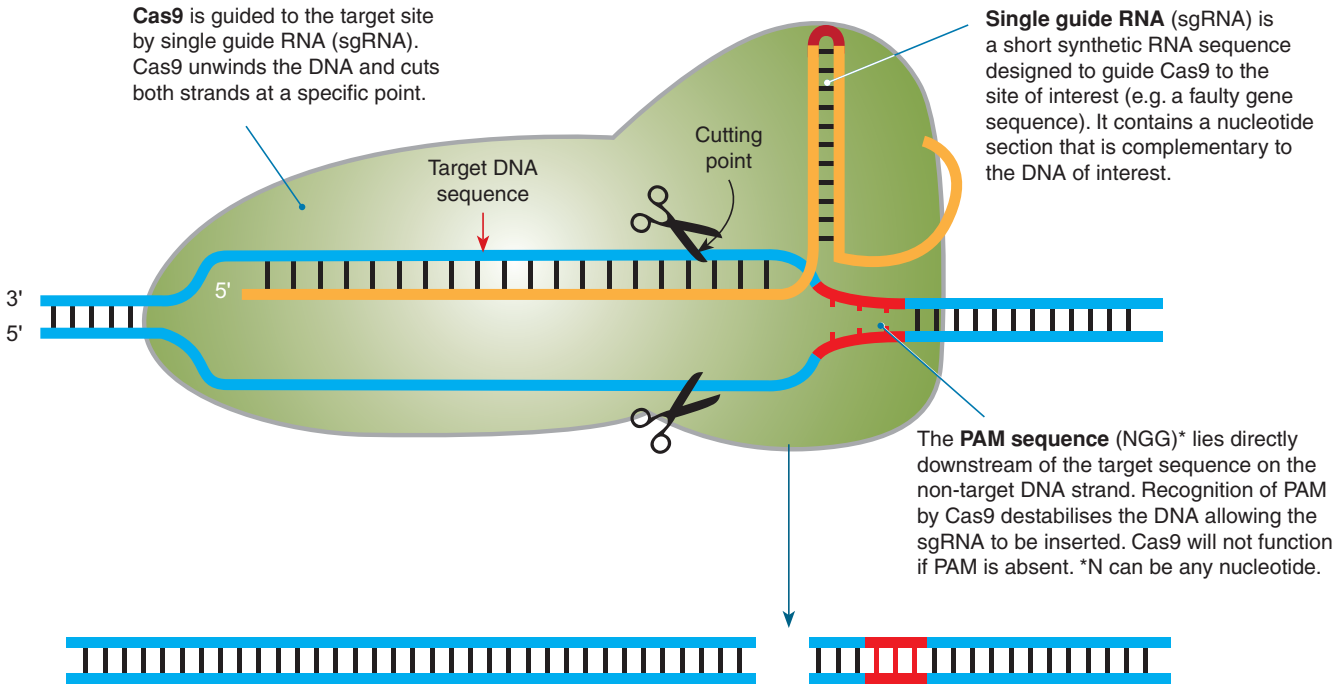
2. Describe the ethical and medical issues surrounding reproductive technologies:

ISBN: 978-1-98-856655-9

127 Future Directions in Biotechnology

Key Idea: CRISPR is a complex made up of a Cas9 endonuclease and sgRNA. The CRISPR complex cuts DNA at very specific sequences and can be used to edit genes.

CRISPR-Cas9 (shortened to CRISPR and pronounced crisper) occurs naturally in bacteria, which use it to edit the DNA of invading viruses. It is an endonuclease complex, i.e. it cleaves the phosphodiester bond in DNA. CRISPR is able to target specific stretches of DNA and edit it at very precise locations. Two key components are required for CRISPR to work: an RNA guide that locates and binds to the target piece of DNA and the Cas9 endonuclease that unwinds and cuts the DNA. The technology has potential applications in correcting mutations responsible for disease, switching faulty genes off, adding new genes to an organism, or studying the effect of specific genes. It represents a major advance because it allows precise and efficient gene editing.



The cut DNA can be repaired using one of the following methods:

Gene knock in 'gene editing'

A new DNA sequence is inserted into the DNA break. For example allows a faulty gene sequence can be replaced with the correct sequence to restore normal gene function.

Gene knock out "gene silencing"

Errors occur as the cell's normal repair mechanisms mend the broken DNA, causing the insertion or deletion of bases. The resulting frame-shift mutation changes the way the nucleotide sequence is read, either disabling gene function or producing a STOP signal. This technique can be used to silence a faulty gene.

1. What are the roles of the following, in CRISPR gene editing:

   (a) Cas9? _____

   (b) sgRNA? _____

2. Outline two ways that CRISPR can be used to edit genes: _____

   _____

   _____

   _____

3. What benefits are offered by CRISPR technology? _____

   _____

   _____

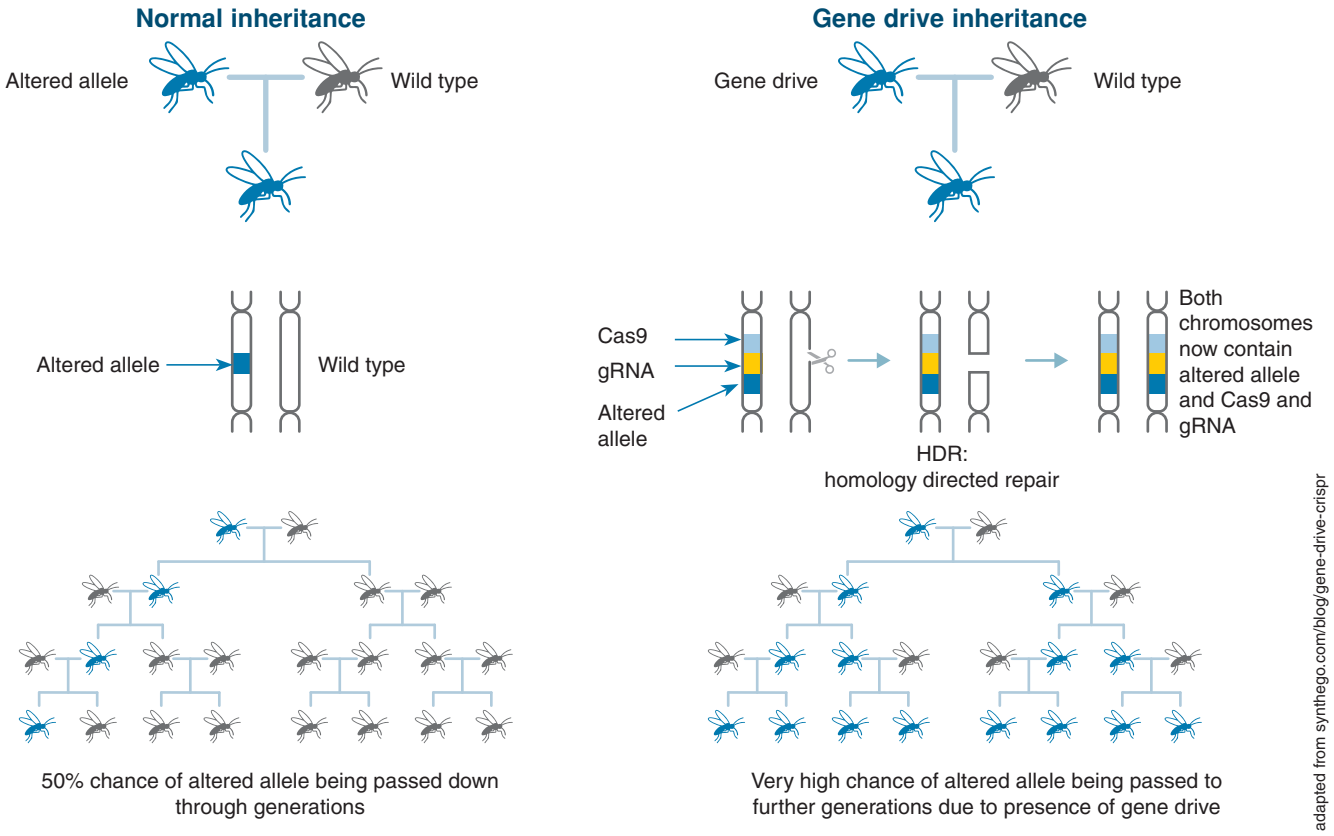
ISBN: 978-1-98-856655-9

Gene drives

CRISPR technology could be used to develop gene drives. Genes edited by the use of CRISPR are driven through a population of organisms at a higher rate of inheritance than by traditional Mendelian inheritance rates. Gene drives have been proposed as a way to potentially eliminate pests such as mosquitoes, that carry malaria, as well as other pest species.

Gene drives use homology directed repair, which is a naturally occurring nucleic acid repair system:

- The normal gene sequence in an organism is replaced with a drive allele containing CRISPR-Cas9 and guide RNA (gRNA) genes.
- The transgenic organism is allowed to breed with wild types.
- During reproduction the drive gene is copied onto the homologous chromosome during the early zygote stage via a homology directed repair system.
- Both chromosomes now contain the edit. In this way, the edit will be passed through to future generations.



Potential uses of gene drives

Grant Singleton CSIRO CC3.0

Eliminate disease – mosquitoes are responsible for the transmission of many diseases that have huge impacts on human health. Diseases such as malaria, Dengue fever and Zika virus can cause severe illness, or death. By using a gene drive targeting the 'doublesex' fertility gene, which is resistant to mutations, females cannot bite and don't lay eggs. Effectively, populations of mosquitoes could be wiped out.

Eliminate invasive rodents – scientists at the University of Adelaide are researching the possibility of using gene drive technology as one of a range of measures to control the impacts of mice. Recent population spikes of pest mice in parts of Australia devastated farm crops and had a huge impact on rural communities. One of the research team's proposed approaches is to use a gene drive to make female mice infertile. If successful, the technology could be used to target other pests such as rabbits and rats.

Save endangered species – a large number of the world's endangered species live on islands and one of the main threats to their survival is invasive rodents. Rats and mice often arrived on islands on ships and, with no natural predators, quickly expanded their populations. By eating young animals and birds' eggs they have reduced numbers of existing insects, animals, and birds. Until now, the only option has been heavy use of rodenticides which can also kill non-rodent native species. An alternative would be to use a gene drive to spread infertility throughout rodent populations.

ISBN: 978-1-98-856655-9

Potential uses of genetic technology

Genetic technology has many potential applications for helping us. Some some ways that biotechnology could, or is already improving our world are listed below.

- **Medical**: vaccinations can be rapidly developed for emerging diseases, as has happened in response to the Covid-19 pandemic.
- **Environmental**: biotechnology could be used to find ways to break down plastics that are a global pollutant.
- **Agricultural**: new healthier crops and livestock could be produced to feed the world's increasing population.
- **Industrial applications** could generate, new, more efficient ways of producing food such as microalgae.
- **Big bio-data** and machine learning can be used to refine precision medicine, which relies on large data-sets.
- **Synthetic biology** has been used to engineer organisms to have new, useful abilities. Microbes can be engineered that clear pollutants from water; crops can be engineered to produce vitamins, e.g. golden rice.
- **Gene and genome engineering** technology could be used to cure genetic diseases by editing harmful genes.

4. Describe how a gene drive can cause most of the offspring to carry the edited gene: _____

5. How can biotechnology be used to help feed the world's growing population? _____

6. How has biotechnology helped the world to deal with the Covid-19 pandemic? _____

7. (a) Which genetic technology can be used to edit harmful genes? _____

   (b) Explain how this technology works: _____

ISBN: 978-1-98-856655-9

128 The Potential Benefits of Genetic Technologies

Key Idea: Genetic technologies have the potential to make our society healthier, provide us with cheaper, more nutritious foods, and improve crop production.

Use of genetic technologies has the potential to benefit our society in many more ways than it does currently. In healthcare, medicines can be tailored to people's specific needs, and produce donor organs for humans in other animals. In agriculture, genetic technologies reduce our reliance on chemical fertilisers, leading to both healthier conditions for farm workers and more efficient crop production. Production of engineered crops that need less water puts less demand on our waterways and creates healthier soils. As the human population continues to increase and the demand for animal protein grows, genetic technology can be used to produce fast growing animal protein sources.

Feeding and clothing the world

Golden bananas for Africa

- The East African, highland cooking banana is a staple food of many East African nations. However, it has low levels of micronutrients, particularly pro-vitamin A. Lack of vitamin A can affect brain development and also weaken the immune system. Vitamin A deficiency is responsible for the deaths of hundreds of thousands of children worldwide, every year, and is also responsible for high levels of blindness. Scientists in Queensland are researching the introduction of genes into bananas that will increase the level of pro-vitamin A that the fruit produces, to improve health outcomes for the people who rely on it as a staple food-source.

Healthier DHA canola oil

- Long chain polyunsaturated fatty acids, known as omega 3 oils, are essential for our health and are normally obtained by eating oily fish or from taking dietary supplements that contain fish-oil. Genes encoding for the omega 3 oil, docosahexanoic acid (DHA), have been successfully placed into canola by scientists. In Australia, a large amount of canola oilseed is grown and it is of great economic importance. Most Australians do not consume the recommended daily amount of omega 3 oils so incorporating DHA into a commonly used food would have clear public health benefits. An increase in consumption of omega-3 oils would reduce cardiovascular disease.

Insect resistant cotton

- Cotton that is resistant to attack by the cotton bollworm, *Helicoverpa* spp., can be grown with far less insecticide than traditional cotton plants. *Bacillus thuringiensis* is a bacterium that produces crystal-like proteins that can kill selected insects by binding to receptors and rupturing mid-gut cells. If this gene for protein production is put into the cotton genome, the plant produces these same Cry-proteins and killing harmful insects that fed on the crop. Cotton containing this gene is known as Bt cotton. By growing Bt cotton, Australia has greatly reduced its use of insecticides. This has led to improved soil quality, reduced cotton production costs, and created a safer working environment for workers.

1. Describe a way that genetic technology could be used to produce healthier food for humans:

2. Why does producing insect resistant cotton lead to a healthier working environment for farmers?

ISBN: 978-1-98-856655-9

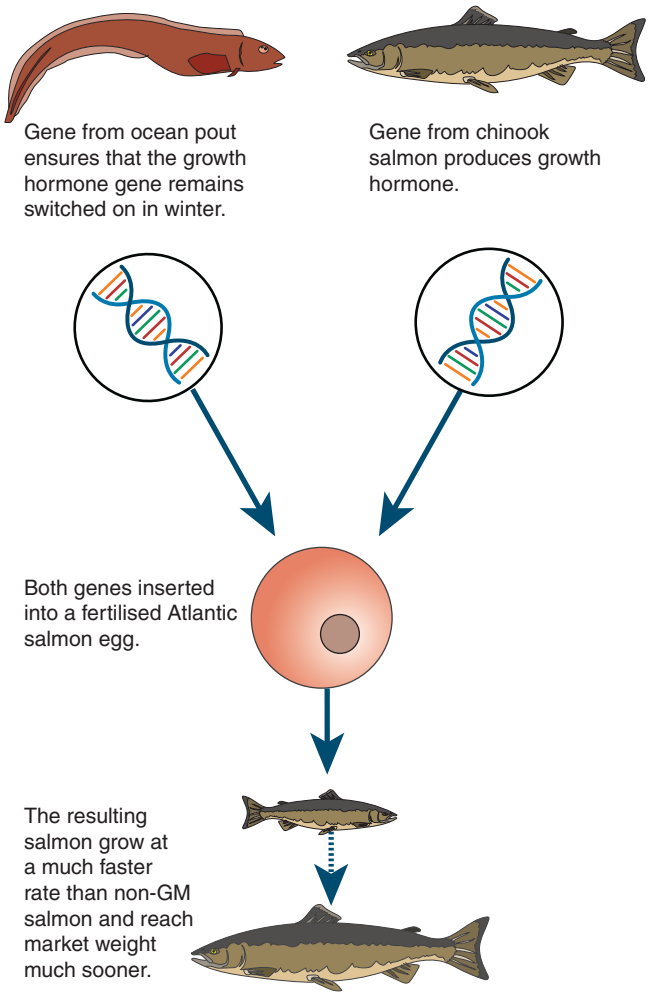
GM salmon - a world first

A new, genetically modified fast-growing Atlantic salmon called AquAdvantage is the first genetically engineered animal to be approved for human consumption. Atlantic salmon is a popular and healthy food. However, a combination of over-fishing, pollution, habitat deterioration, and disturbance of migration routes have caused wild populations to decline. Farmed fish are now the main source of salmon as a human food. It is estimated that seafood consumption could increase dramatically as people look to eat a healthier diet and farmed fish production will have to expand to meet human needs.

Photo above: A salmon farm in Norway

- A gene from the fast growing pacific chinook salmon switches on production of extra growth hormone.
- A gene from the ocean pout keeps the growth gene 'switched on' during winter months when the Atlantic salmon would not normally grow.
- Together, these genes are placed into fertilised Atlantic salmon eggs. As the embryo grows, the added genes speed up the fish growth.
- The resulting fish reach 2-3kg in weight in 18-24 months, compared to 3 years for non-GM salmon.
- Fish produced are all selected to be sterile females, ensuring that the gene cannot escape into the wild population.
- Fish can be produced in inland fish farms, close to cities.

Development of GM salmon



3. Why might it be beneficial to fish farmers to produce market weight salmon in a short space of time? _____

_____

_____

_____

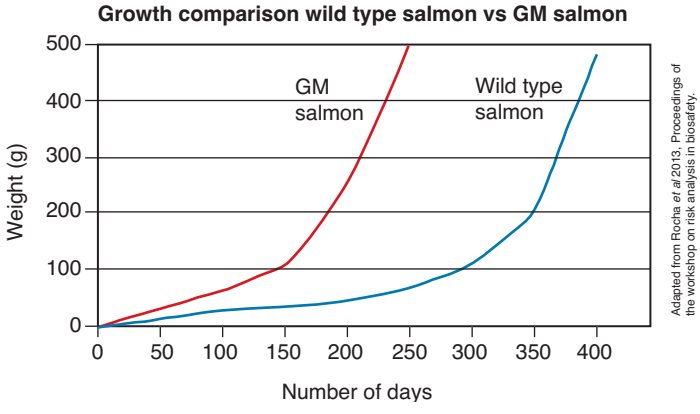
4. The graph on the right shows the growth rate of a wild type salmon compared to an AquAdvantage, GM salmon. Approximately how many days does it take for each type of salmon to reach:

   (a) 200g? _____

   _____

   (b) 500g? _____

   _____



Adapted from Rocha *et al* 2013. Proceedings of the workshop on risk analysis in biosafety.

5. What steps did the researchers take to ensure GM salmon do not interbreed with wild populations?

_____

_____

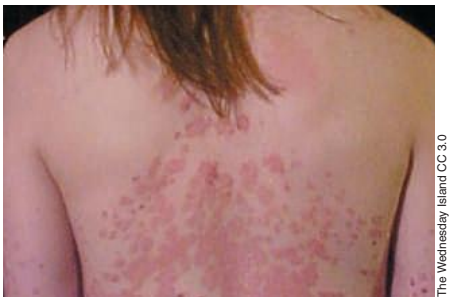
ISBN: 978-1-98-856655-9

A healthier society

Using biotechnology in medicine has many benefits for society including:

- Cheaper, more widely available drugs.
- Better quality of life for people suffering from certain conditions.
- Lower cost of healthcare for society, which has economic benefits.

New vaccine development – the ability to make new vaccines quickly for both existing and emerging diseases such as Covid-19 has huge benefits for society. By vaccinating against diseases, healthcare costs over the long term can be drastically reduced as fewer people will need complicated, expensive treatment for disease.

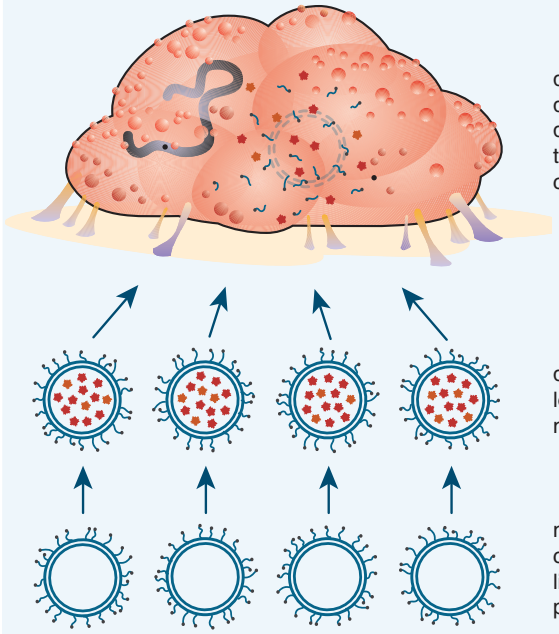


Enhanced diagnosis and treatment for autoimmune disease – the body's defences can turn against the body itself and attack it as though it were an invading pathogen. Biotechnology can be used to develop better treatments for conditions such as Graves' disease, psoriasis, vitiligo and Addison's disease, amongst others, leading to improved quality of life for sufferers.

Genome analysis for personalised medicine – by examining a person's genome, healthcare treatments can be tailored towards a person's unique genetic make-up. This means that more effective drugs can be used and those that cause unpleasant or harmful side-effects can be avoided. We can also determine whether a person is susceptible to a particular disease.

Nanomedicine

- Nanoparticles are tiny molecules of 1-100 nanometres (nm)



- In medicine, ensuring that drugs reach the target area of the body is a challenge for drug developers. Many drugs are non water-soluble, which poses particular problems in the body.
- Chemotherapy is often used as part of a cancer treatment plan but has unpleasant side effects including tiredness, nausea, and hair loss. These effects are caused by the chemotherapy drugs targeting non-cancer cells. By delivering drugs straight to cancer cells, many of these unpleasant side effects can be minimised.
- By 'loading' biological nanoparticles with appropriate drugs, and specific targeting markers, drugs can be delivered straight to the correct region of the body. For example, many cancer cells over-express specific proteins. These can be targeted by nanoparticles to ensure they only deliver the drugs to these cells. Other chemical groups can be attached to the nanoparticles, including a stimulus system, to ensure it releases the drugs at the appropriate time.
- Nanoparticles are being explored in many areas of medicine, for example, precise insulin delivery for diabetics, treating Alzheimer's disease and Parkinson's disease, also cardiovascular disease, including blood pressure regulation, and respiratory diseases, including asthma.

6. How could using nanoparticles to deliver chemotherapy drugs reduce unpleasant side effects for cancer patients?

ISBN: 978-1-98-856655-9

129 Genetic Technologies and Earth's Biodiversity

Key Idea: Biodiversity is a measure of all the alleles that exist. By altering genes, technology can alter biodiversity.

Having a wide range of alleles within a population of organisms makes it more resilient. It increases the likelihood of the population surviving disease outbreaks or catastrophic events such as fires and drought. Genetic technology can be used to widen a gene pool, for example, by adding new genes to a population, or editing existing ones. However, if those organisms with altered genomes are used to populate large areas of land then we decrease overall diversity in a given area. We can use genetic technology to enhance numbers of endangered organisms, or potentially, to bring extinct species back into existence, both of which would increase biodiversity.

What is biodiversity and why is it important?

- Biodiversity is the variety of all life on Earth, including the animals, plants, and micro-organisms present in ecosystems. We can think of biodiversity as all the alleles that exist. We can look at biodiversity in small areas, such as one ecosystem, or on a much bigger national, or global scale.
- High levels of biodiversity are beneficial. High genetic diversity allows organisms to react to changes within their environment, survive disease outbreaks, and be more resilient to climate change. Farming almost always decreases biodiversity.

Thomas Schoch cc 2.5

This Australian rainforest displays high levels of biodiversity. It supports a wide variety of plants, insects, animals, and micro-organisms.

This monoculture of soy shows little diversity in the life it supports. The soil microbiota is also likely to lack diversity.

Human activities and biodiversity

- Humans have caused significant loss of biodiversity in many parts of the world through the introduction of invasive species, pollution, exploitation of the environment by mining and deforestation, and also by activities leading to climate change.
- Genetic technology has the power to change biodiversity. By using the technology that we possess to alter the genomes of organisms we can, in some ways, increase biodiversity. However, if we 'mass produce' those altered organisms in one area, then we will decrease the biodiversity in that place.

1. Describe three ways in which humans have altered biodiversity around the world: _____

2. Why is it important for a population of organisms to have good genetic diversity? _____

ISBN: 978-1-98-856655-9

Genetic technology has both positive and negative effects on biodiversity

- The effect of different genetic technologies on biodiversity is not straightforward. While some technologies, such as cloning, decrease biodiversity (imagine if all farms had identical cows or sheep!), others can be thought of as increasing biodiversity.

Techniques that increase biodiversity

- CRISPR could be considered as increasing biodiversity by introducing new gene combinations into organisms.
- Transgenic plants such as Bt cotton, or animals such as AquAdvantage salmon, carry genes that would not normally be found in the population. In this respect, biodiversity has been increased, as the gene pool has been widened.
- Because some transgenic plants reduce reliance on insecticides, they could increase insect biodiversity in an area. Instead of all insects being wiped out by non-selective insecticides, those insects that do not attack the crop plants will survive.
- Artificial insemination could potentially increase biodiversity by introducing new gene types into a herd of cattle, widening the gene pool on a farm.

Techniques that decrease biodiversity

- CRISPR could potentially be used to eradicate undesirable organisms such as mosquitoes or rodents and, therefore, decrease biodiversity.
- Transgenic plants such as Bt cotton are grown in vast quantities over large areas which greatly reduces biodiversity in these places.
- Artificial insemination could be used to ensure that multiple cows have calves sired by the same bull, both nationally and internationally. This could decrease the gene pool of cattle bred on farms
- Cloning organisms by making multiple copies with identical genomes reduces biodiversity.

3. Why can the activity of taking genes from one organism and placing them into a different species (transgenics) be considered as increasing biodiversity?

4. Describe a way in which CRISPR could ultimately be used to reduce biodiversity:

5. Explain why artificial insemination has the potential to both increase and decrease biodiversity:

6. Give an example of a genetic technology that decreases biodiversity. Explain why the decrease occurs, and why it puts the population of organisms at risk:

7. In small groups, choose one specific genetic technology and research its effects on biodiversity in a specific ecosystem. Present your group's findings to the rest of the class as a poster:

ISBN: 978-1-98-856655-9

Using technology to protect biodiversity

The range of possibilities and problems that genetic engineering and biotechnologies could be applied to is vast. Many are limited by our understanding of the biochemical pathways controlled by genes. However, as we understand more about these, the limitations of genetic engineering and biotechnology becomes less.

- Biotechnologies could be used to:
  - Introduce useful genes into plants that we can eat but which don't work well as crop plants grown in large-scale agriculture.
  - Expand the genetic diversity of current plants to ensure they are less susceptible to disease.
  - Engineer crop plants to be more resistant to drought to help cope with drier climate and to reduce our reliance on irrigation, which can negatively affect waterways.
  - Help with coral conservation efforts by using CRISPR to establish which coral genes are responsible for the organism being affected by heat stress.
  - Help protect endangered species by using gene technology to eradicate pests species that harm them by competing for food or killing their young.

Micropropagation

Plant micropropagation techniques can be used to make many multiple copies of threatened plants and can be used as stock material, in case of extinction threat. This technique can be carried out all year and is not dependent on seasonal timing for natural plant reproduction.

Gene banks

Preserving genes in gene banks can ensure against loss of genetic diversity in sudden events such as floods and/or fires, which could otherwise cause a gene bottleneck. Seed banks and frozen genetic material such as gametes, embryos, and cell lines can be used in conservation efforts.

De-extinction

Bringing back organisms that are extinct would increase biodiversity. However, such activities could counter efforts to protect endangered species. Money spent on researching technology to bring back extinct species could take away from money that is being used to protect endangered species.

8. How can gene banks help preserve biodiversity? _____

9. Describe how genetic technology could help protect endangered species: _____

10. Explain why de-extinction could undermine conservation efforts for endangered species: _____

11. Explain how we can use genetic technologies to make our food supply more resilient to climate change:

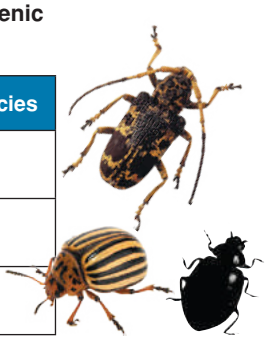
ISBN: 978-1-98-856655-9

130 Chapter Review: Did You Get It?

1. Examine the data below for a hypothetical experiment where researchers compared the effect of growing transgenic cotton on two species of invertebrates.

Comparison of invertebrate populations in uncultivated land; crop of transgenic cotton; and crop of insecticide-sprayed, non-transgenic cotton

	Number of ant species	Number of beetle species
Uncultivated land	27	22
Non transgenic cotton crop, sprayed with insecticide	8	6
Bt cotton crop	10	14



(a) Plot the data on the grid below, using a suitable format for comparison:

(b) Which area had the fewest invertebrates? _____

(c) Suggest why beetle species were not reduced as much as ant species in the Bt cotton crop: _____

_____

_____

_____

(d) What other data could the researchers have collected to improve their study? _____

_____

_____

2. Why might a couple prefer to know that one of them carries a gene for a particular disease? _____

_____

_____

_____

_____

ISBN: 978-1-98-856655-9

CHAPTER
8

Genetic Technologies

Activity number

Key terms

Agrobacterium tumefaciens
artificial selection
biodiversity
clone
DNA ligase
endonuclease
genetically modified organism (GMO)
in-vitro fertilisation
micropropagation
plasmid
recombinant DNA
reverse transcriptase
somatic cell nuclear transfer (SCNT)
transgenic

Inquiry question: *Does artificial manipulation of DNA have the potential to change populations forever?*

Reproductive technologies

Key skills and knowledge

Toni Barros cc 2.0

USDA

Using recombinant DNA

Key skills and knowledge

Biotechnology and society

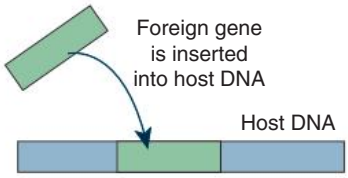
Key skills and knowledge

131 An Overview of Current Genetic Technologies

Key Idea: DNA manipulation alters an organism's DNA either by adding new DNA or editing the existing DNA.

DNA manipulation, also called genetic engineering, involves the direct manipulation of an organism's genome using biotechnology. This can be achieved by introducing new DNA into an organism or by editing its existing DNA. DNA manipulation aims to repair damage or to produce improved or novel organisms with specific traits. Genetic engineering has wide applications in food technology, industry, agriculture, environmental cleanup, pharmaceutical production, and vaccine development. Organisms that have had their DNA altered are called genetically modified organisms (GMOs).

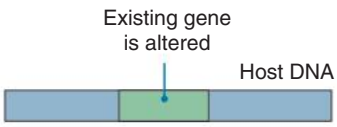
How are genetically modified organisms produced?



Add a foreign gene

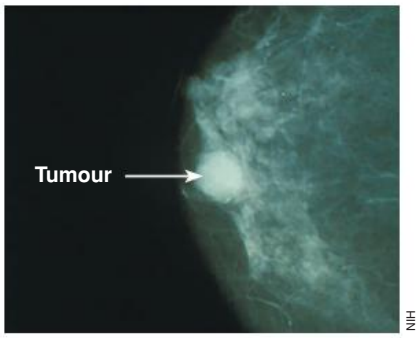
A novel gene (foreign to the recipient) is inserted from another species. This will enable the GMO to express the trait encoded by the new gene. Organisms genetically altered in this way are referred to as **transgenic**.

Human insulin, used to treat diabetic patients, is produced by inserting the insulin gene into bacteria and yeast.

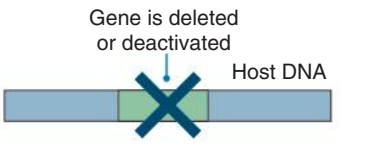


Alter an existing gene

An existing gene may be altered to make it express at a higher level, e.g. growth hormone, or in a different way, e.g. in tissue that would not normally express it. The technique may provide a way to fix a malfunctioning gene.



Gene editing technologies such as CRISPR are being explored to treat breast cancer (above) and sickle cell disease.



Delete or 'turn off' a gene

An existing gene may be deleted or deactivated (switched off) to prevent the expression of a trait, e.g. the deactivation of the ripening gene in tomatoes produced the Flavr-Savr tomato.

Manipulating gene action is one way to control processes such as ripening in fruit so that it stays fresher for longer.

1. (a) What is DNA manipulation? _____

(b) Using examples, discuss the ways in which an organism may be genetically modified: _____

2. Describe some of the applications of DNA manipulation: _____

ISBN: 978-1-98-856655-9

132 Selection and Population Change

Key Idea: Artificial selection is the process of breeding together organisms with desirable qualities, e.g. high milk yield, to ensure that the trait is reliably passed on to the next generation.

Although it does not directly alter the DNA of an organism, **artificial selection** (or selective breeding) is a potent technique for ensuring that the best genes appear in plants or animals. In artificial selection, humans select organisms with desirable traits and breed them together so that the trait appears in the next generation. The process is repeated over many generations, until the characteristic becomes common. Artificial selection often uses reproductive technologies such as artificial insemination, to ensure that the desirable traits of one male can be passed to many offspring. This increases the rate at which the desirable trait is passed to progeny. There are problems associated with artificial selection. The gene pool becomes more constrained and some alleles may be lost. A reduction in genetic diversity decreases the ability to redirect a population's traits if circumstances change (such as breeding cattle that produce less methane).

The origins of domestic dogs

All breeds of dog are members of the same species, *Canis familiaris,* and provide an excellent example of selective breeding. The dog was the first domesticated species and, over centuries, humans have selected for desirable traits so extensively that there are now more than 400 breeds of dogs. Until very recently, the grey wolf was considered to be the ancestor of the domestic dog. However, recent (2015) genetic studies provide strong evidence that domestic dogs and grey wolves are sister groups. They shared a now extinct, wolf-like, common ancestor which gave rise to the dog before the agricultural revolution, 12,000 years ago. Based on genetic analysis, four major clusters of ancient dog breeds are recognised. Through artificial selection, all other breeds are thought to have descended from these clusters.

1: Older lineages
The oldest lineages, including Chinese breeds, basenji, huskies, and malamutes.

2: Mastiff-type
An older lineage that includes the mastiffs, bull terriers, boxers, and rottweilers.

3: Herding
Includes German shepherd, St Bernard, borzoi, collie, corgi, pug, and greyhound.

4: Hunting
Most arose in Europe. Includes terriers, spaniels, poodles, and modern hounds.

Modern dog breeds exhibit a huge variety of physical and behavioral phenotypes. Selective breeding has produced breeds to meet the specific requirements of humans.

Problems with selective breeding

Selection for a desirable phenotype can lead to a consequential emphasis of undesirable traits, often because genes for particular characteristics are linked and selection for one inadvertently selects for the other. For example, the German shepherd is a working dog, originally bred for its athleticism and ability to track targets. However, in German shepherds bred to meet the specific appearance criteria of show dogs, some traits have been exaggerated so much that it causes health issues. The body shape of the show German shepherd has been selected for a flowing trot and has a pronounced sloping back. This has resulted in leg, hip, and spinal problems. In addition, selective breeding has increased the incidence of some genetic diseases such as epilepsy and blood disorders.

1. (a) What is artificial selection? _____

_____

_____

(b) What are the advantages of artificial selection? _____

_____

_____

2. What effect would artificial selection have on the genetic diversity of a population? _____

_____

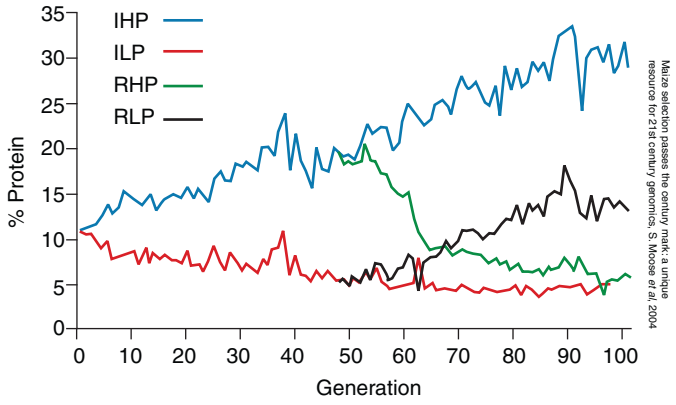
_____

ISBN: 978-1-98-856655-9

The effect of selection of populations

- In 1896, researchers in Illinois began an artificial selection experiment with maize (*Zea mays*). The experiment ran for over 100 generations. Starting with a single strain of maize, the researchers selected for high protein content in the maize seeds (Illinois High Protein: IHP), and low protein content (Illinois Low Protein: ILP).
- After 48 generations they reversed their selection in some of the plants in each strain to produce two new strains (Reverse Low Protein: RLP, and Reverse High Protein: RHP)

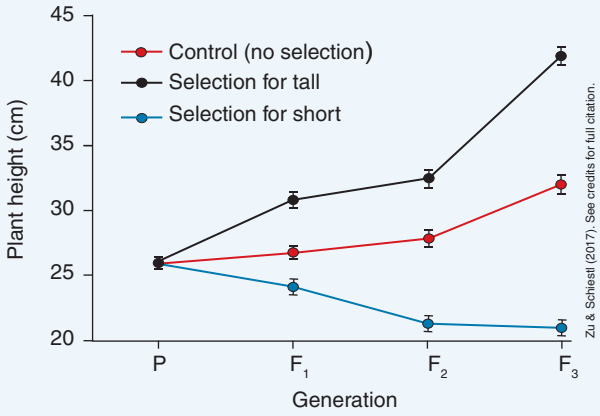
Protein in maize



Maize selection passes the century mark: a unique resource for 21st century genomics, S. Moose *et al.* 2004

- A similar experiment was carried out on *Brassica rapa* (field mustard) by a different group of researchers in 2016. They selected for tall and short plants. Although the experiment only lasted three generations, the difference in height between the original and F_3 generations is easy to see.
- Species in the *Brassica* genus respond rapidly to selection as indicated by the plot below. This makes it easy to produce new varieties rapidly.

Selection for height in *Brassica rapa*



Zu & Schiestl (2017). See credits for full citation.

USDA

The number of apple varieties available now is a fraction of the many hundreds grown a century ago. Apples are native to Kazakhstan and breeders are now looking back to this centre of diversity to develop apples that are resistant to the bacterial disease that causes fire blight.

In 18th-century Ireland, potatoes were the main source of food for about 30% of the population, and farmers relied almost entirely on one very fertile and productive variety. That variety proved susceptible to the potato blight fungus, which resulted in a widespread famine.

Corn and other crop species are often inbred to maximise homozygosity for specific traits in parental lines. These parental lines are then bred together to maximise heterozygosity in the offspring. This has the effect of producing vigorous growth in the offspring (called hybrid vigour).

3. Describe the effect of artificial selection on maize: _____

4. Describe the data shown in the plot of height selection in *Brassica rapa,* above: _____

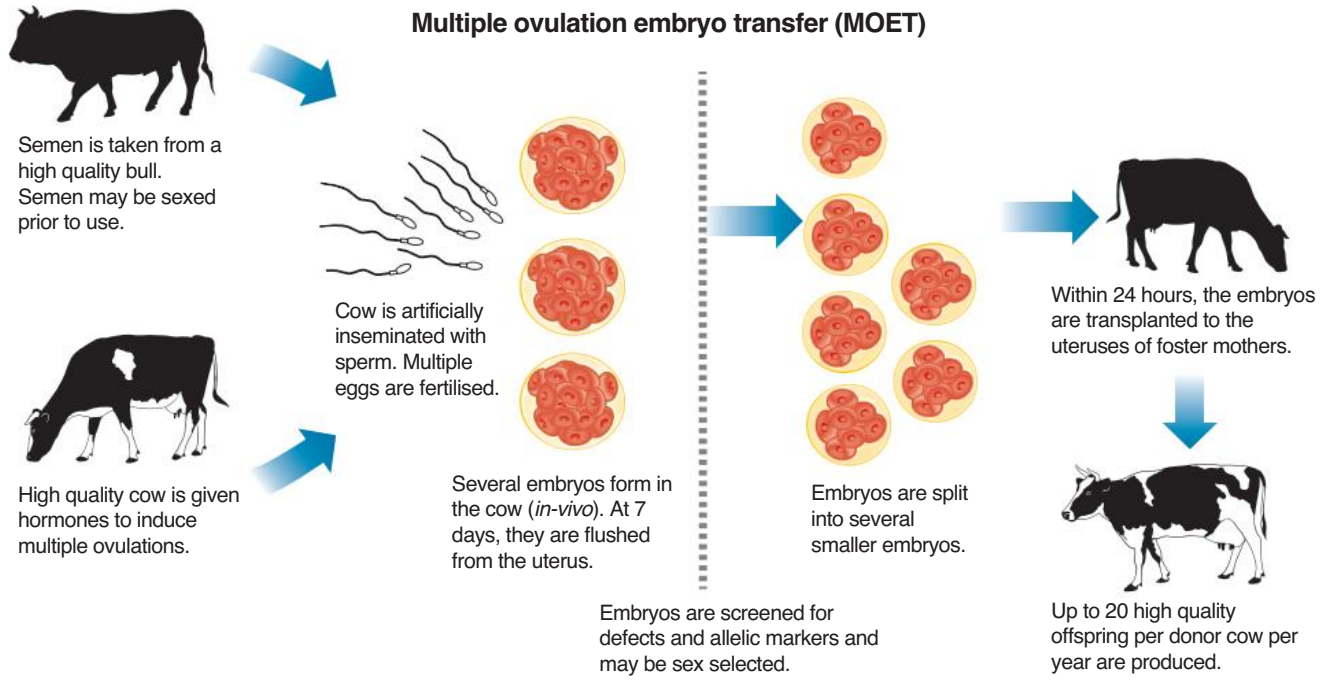
5. Why is it important to maintain the biodiversity of wild plants and ancient farm breeds? _____

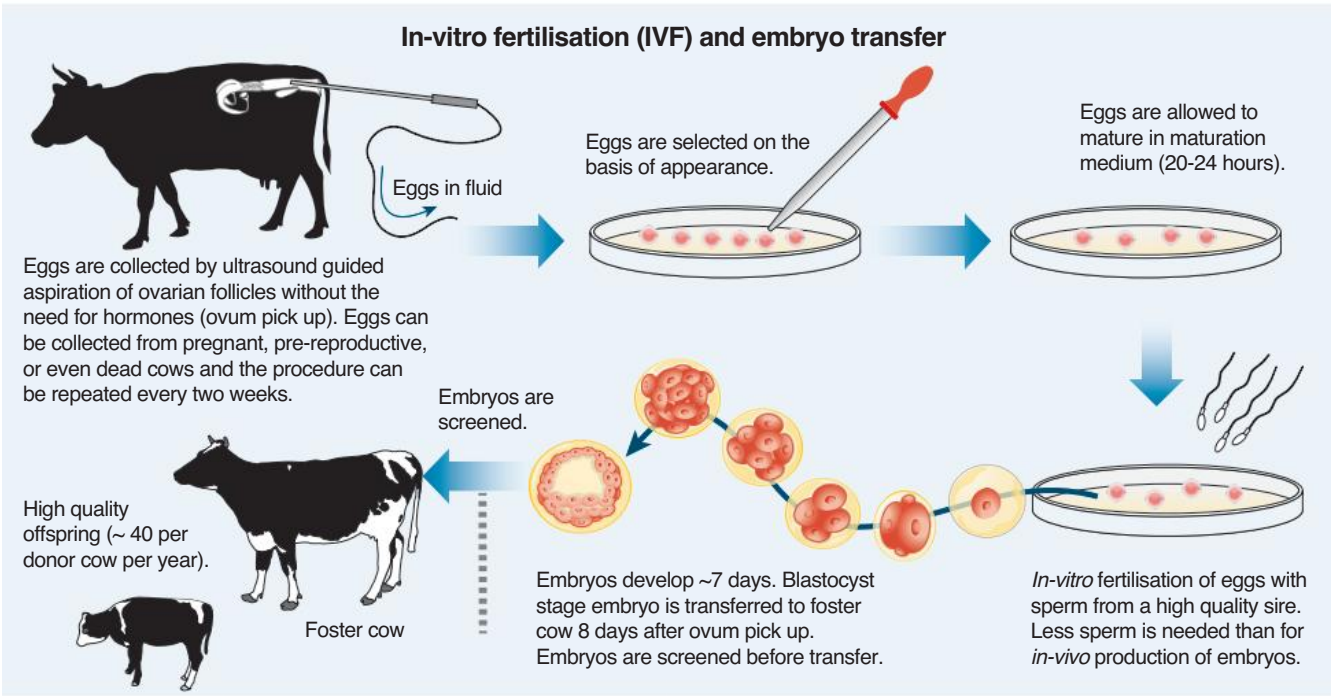
ISBN: 978-1-98-856655-9

133 Reproductive Technologies in Agriculture

Key Idea: Rapid production of high quality livestock is possible using reproductive techniques such as artificial insemination (AI), embryo transfer (ET), and *in-vitro* fertilisation (IVF).

The selection and breeding of cattle for dairy and beef production is a huge industry. Breeders have been assisted by rapid advances in reproductive technologies to capitalise on the reproductive potential of superior individuals. This has improved the genetics of herds (genetic gain) more rapidly than would occur by conventional selection methods. Artificial insemination is now a well established technology and has allowed breeders to maximise offspring from superior sires to improve their herds. More recently, *in-vitro* fertilisation and embryo transfer (ET) technologies have accelerated these genetic gains. ET also permits screening and embryo selection technologies to be used to select for sex and superior genetic profiles, and screen out defective progeny. IVF and ET are currently used to improve herd genetics in other livestock too, including goats and horses.



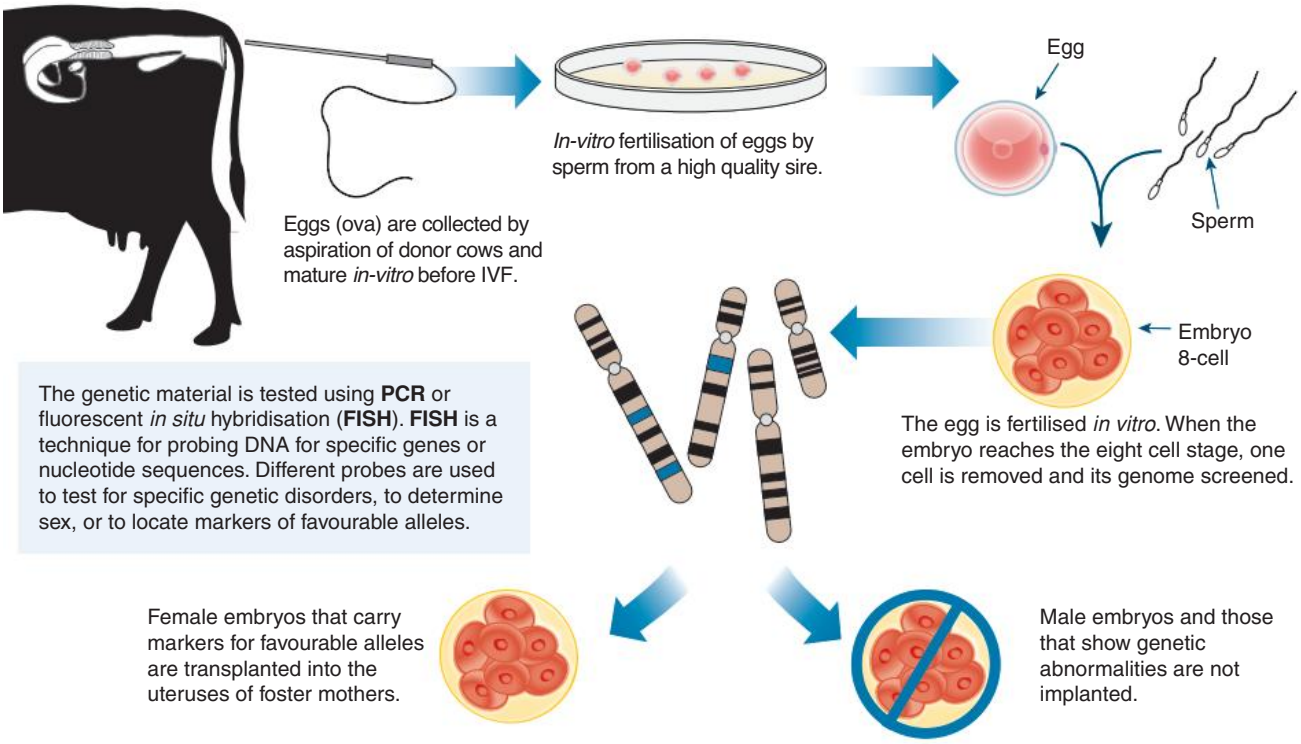


1. Compare and contrast the biological features of MOET and embryo transfer after IVF in cattle: _____

ISBN: 978-1-98-856655-9

Pre-implantation genetic diagnosis (PGD) and embryo selection in cattle

- Genetic testing of embryos prior to their transfer to the uterus (PGD) is an established feature of human reproductive technology as it enables parents to select only healthy embryos for transfer. Technical advances in PGD in cattle embryos is now allowing the procedure to be integrated into embryo transfer programmes in the dairy and beef industries too. PGD allows embryos to be selected on the basis of their allelic makeup, as identified by markers. It can be used to select (or reject) embryos on the basis of their sex or valuable production traits (using Marker Assisted Selection).



2. How do IVF and embryo transfer technologies increase the rate of genetic gain relative to natural breeding practices (bull with a herd), and artificial insemination of individuals in a herd?

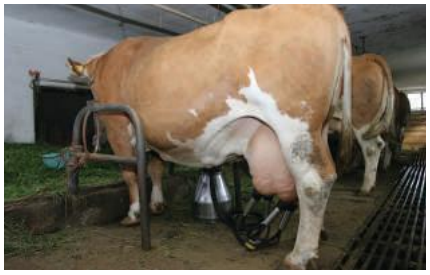
3. Young male calves (bobby calves) are often seen as a 'waste product' of the dairy industry and most go to slaughter. How could techniques such as sexing of sperm and embryo selection improve animal welfare in the dairy industry?

4. Discuss the genetic consequences of the rapid genetic gains in one direction, achieved by IVF and ET technologies:

ISBN: 978-1-98-856655-9

- Most of the economically important traits in dairy cattle are expressed only in females, but the main opportunity for selection is in males. Selection of the best bulls, combined with their worldwide use through artificial insemination and frozen semen, has seen a rapid genetic gain (the increase in performance as a result of genetic changes) in dairy cattle since the 1970s. Bulls are assigned breeding values based on the performance of their daughters and granddaughters. In this way, the bulls and cows with the best genetics can be selected to produce the next generation.
- Beef breeds tend to show important traits in both males and females as desirable traits include high muscle development and growth.

Beef breeds: Selection is for large breeds with a high proportion of lean muscle. **Desirable traits**: high muscle to bone ratio, rapid growth and weight gain, hardy, easy calving, docile temperament.



Dairy breeds: Selection is based primarily on high milk production, but good health and fertility are also selected for. **Desirable traits**: high milk yield with good protein and fat content, fast milking speed, docile temperament, good udder characteristics (e.g. good teat placement).

Special breeds: Some cattle are bred for their suitability to climate or terrain. Scottish highland cattle (above) are a hardy, long coated breed and produce well where other breeds cannot thrive.

Artificial pollination

- Artificial pollination is important in developing plant varieties. In plants that have both male and female reproduction parts the male anthers are often removed to prevent self pollination. Pollen is brushed onto the stigma from the anthers of a selected plant. The flowers must be covered to prevent pollinating insects from transferring pollen between plants.
- Artificial pollination is useful in places such as greenhouses, where using insects may be difficult.
- Over the last few decades, the number of honeybees has been in decline. Various factors are involved but they include the use of broad spectrum insecticides.
- It is estimated that bees have an international economic value of about US$150 billion dollars a year. They pollinate about a third of the food we eat and 80% of flowering plants. Not surprisingly, their decline is both a major economic and biodiversity problem.
- One way researchers are preparing for a possible decline in pollinators is in the development of drones that can automatically pollinate flowers.
- Mechanical artificial pollination is not new. Many devices are available to enhance pollination, including hand held dusters, and blowers that mount to quad bikes and tractors.

5. Suggest why selective breeding has proceeded particularly rapidly in dairy cattle: _____

_____

_____

_____

_____

6. (a) Describe some current uses of artificial pollination: _____

_____

_____

_____

(b) Why might artificial pollination become more important in future? _____

_____

ISBN: 978-1-98-856655-9

134 Applications of Plant Micropropagation

Key Idea: Large numbers of genetically identical plants can be grown from a small amount of tissue.

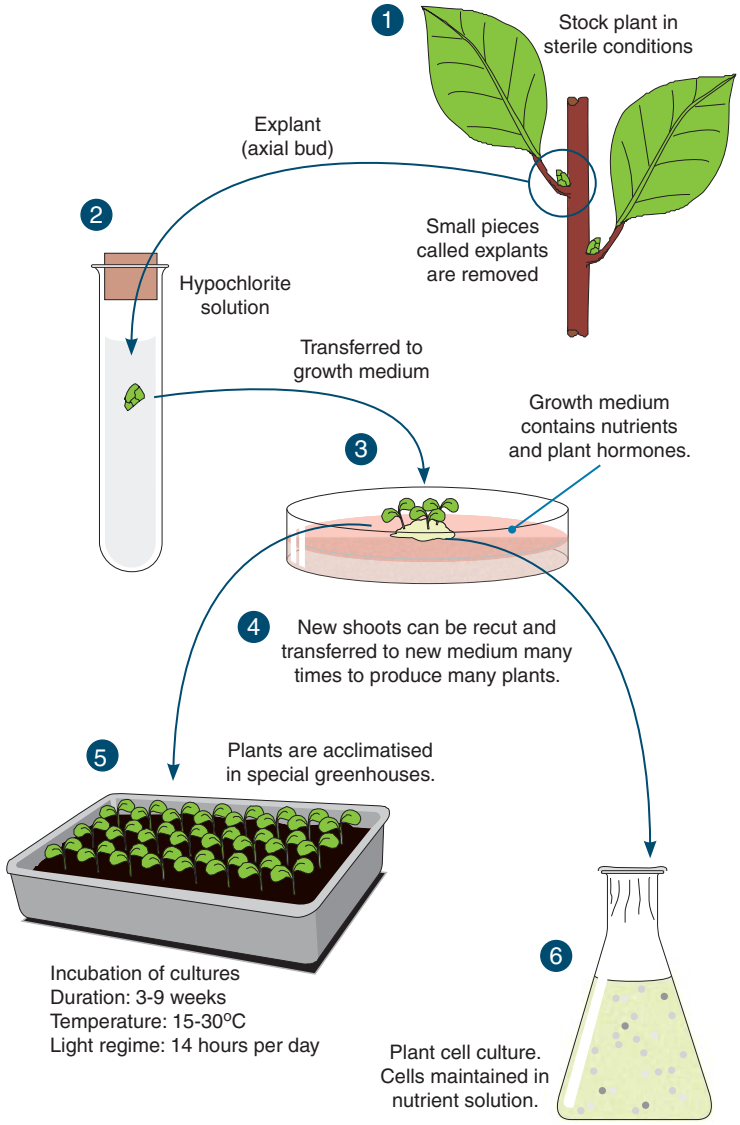
Individual plants vary in their characteristics, even within the same species. From a commercial perspective, uniform plants are easier to manage and handle but when plants are naturally pollinated, they are rarely uniform. Some plants also produce few seeds, making propagation difficult. Genetically identical, uniform plants can be produced by micropropagation techniques. These are useful for crop management, quality control and use in transgenics. Plant micropropagation can also be used in the recovery of endangered species.

Concept 1
Plants can be cut into many pieces, almost all of which have the potential to grow into new plants.

Concept 2
Plants propagated from the same tissues will all have the same genetic make up. They will all be clones.

Concept 3
Different **growth hormones** in plants cause the development of different parts of the plant, e.g. the shoots or roots.

Concept 4
If desirable plants are used for the original tissue then all the plants propagated from them will also be desirable.



Techniques

- Pest free stock plants have small pieces cut (**excised)** from them. These pieces, called explants, may be stem tissue, nodes, flower buds, leaves, or sections of shoot tip meristem.
- The surface of the explants are sterilised using a solution of sodium hypochlorite.
- The explants are transferred to a culture vessel under sterile conditions.
- Growth hormones are added. By changing the concentrations of different hormones, the explants can be made to grow shoots, roots, stems, or an undifferentiated cell mass (callus).
- Plants induced to grow shoots and roots are transferred to growing containers under controlled greenhouse conditions to be acclimatised before planting outside.
- Calluses are mechanically broken up into separate cells and placed into a growth medium, where they can be maintained indefinitely.

Outcomes

Plants propagated in this way may be genetically unstable or infertile, with chromosomes structurally altered or in unusual numbers. The success of tissue culture is affected by factors such as selection of explant material, the composition of the culture media, plant hormone levels, lighting, and temperature. New genetic stock may be introduced into cloned lines periodically to prevent reduction in genetic diversity.

Further applications

Plant tissue culture is extensively used in forestry to produce trees of uniform height and diameter. It has a number of advantages, including the ability to generate large numbers of plants from one explant and rapid propagation of species that have a long generation time or low seed production.

ISBN: 978-1-98-856655-9

1. Explain why micropropagation is a useful method for rapidly producing uniform plants:

2. Explain why many commercial operations such as forestry are interested in micropropagation:

3. Carry out an internet search for the use of micropropagation (tissue culture) in conservation and industry.

   - Identify one plant that micropropgation could be used to help conserve. Explain the reasons why the plant is endangered, and the advantages and disadvantages of using micropropagation in their conservation.
   - Identify one plant where micropropgation is used to help commercial production. Explain how the plant is used commercially, and why micropropagation is used in its commercial growth and development.
   - The **BIOZONE Resource Hub** has some useful links.

4. Why do the stock plants used for micropropagation need to be very carefully selected? _____

ISBN: 978-1-98-856655-9

135 Process and Applications of SCNT

Key Idea: Clones can be made by fusing an empty egg cell with a cell from the organism to be cloned.

Clones produced using traditional embryo-splitting must mature before their phenotype is known. Scientists wanted to speed up the selection process and produce clones directly from a proven phenotype. The technique developed to do this is called **somatic cell nuclear transfer**. It involves returning a somatic (body) cell (from an individual of known phenotype) to a dormant state and then fusing it with an egg cell in which the nucleus is removed. Embryonic development is triggered and the resulting embryo is implanted into a surrogate mother. The primary aim of the new cloning technologies is to provide an economically viable way to rapidly produce transgenic animals with very precise genetic modifications.

Concept 1
Somatic cells can be made to return to a dormant or embryonic state so that their genes will not be expressed.

Concept 2
The nucleus of a cell can be removed and replaced with the nucleus of an unrelated cell. Cells can be made to fuse together.

Concept 3
Fertilised egg cells produce embryos. Egg cells that contain the nucleus of a donor cell will produce embryos with DNA identical to the donor cell.

Concept 4
Embryos can be implanted into surrogate mothers and develop to full term with seemingly no ill effects.

Somatic cell nuclear transfer (SCNT)

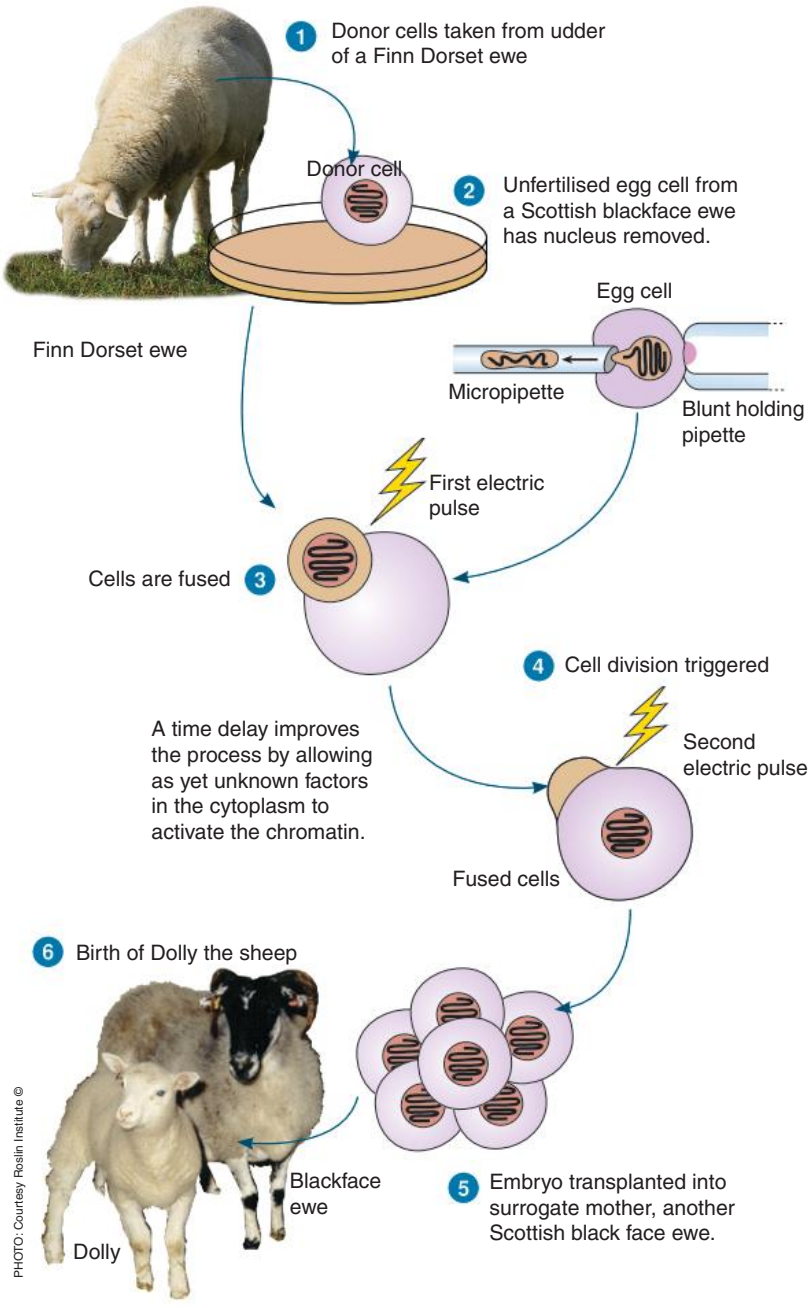


PHOTO: Courtesy Roslin Institute ©

Techniques

- Donor cells from the udder of a Finn Dorset ewe are taken and cultured in a low nutrient media for a week. The nutrient-deprived cells stop dividing and become dormant.
- An unfertilised egg from a Scottish blackface ewe has the nucleus removed using a micropipette. The rest of the cell contents are left intact.
- The dormant udder cell and the recipient denucleated egg cell are fused using a mild electric pulse.
- A second electric pulse triggers cellular activity and cell division, jump starting the cell into development. This can also be triggered by chemical means.
- After six days the embryo is transplanted into a surrogate mother, another Scottish blackface ewe.
- After a 148 day gestation 'Dolly' is born. DNA profiling shows she is genetically identical to the original Finn Dorset cell donor.

Outcomes

Dolly, a Finn Dorset lamb, was born at the Roslin Institute (near Edinburgh) in July 1996. She was the first mammal to be cloned from non-embryonic cells, i.e. cells that had already differentiated into their final form. Dolly's birth showed that the process leading to cell specialisation is not irreversible and that cells can be 'reprogrammed' into an embryonic state. Although cloning seems relatively easy there are many problems that occur. Of the hundreds of eggs that were reconstructed only 29 formed embryos and only Dolly survived to birth.

Further applications

In animal reproductive technology, cloning has facilitated the rapid production of genetically superior stock. These animals may then be dispersed among commercial herds. The primary focus of the new cloning technologies is to provide an economically viable way to rapidly produce transgenic animals with very precise genetic modifications.

ISBN: 978-1-98-856655-9

Advantages and disadvantages of animal cloning

Advantages

- Rapid production of animals with desirable qualities, e.g. high wool quality.
- Applications for production of disease-resistant livestock, conservation (right), or treatment of disease.

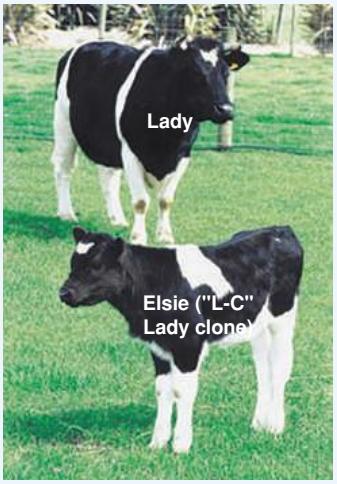
Disadvantages

- SCNT can be inefficient (e.g. many hundreds of attempts were made before Dolly was successfully cloned).
- Many cloned animals are much bigger at birth than their natural counterparts. This is called large offspring syndrome (LOS). Clones with LOS have abnormally large organs, and can suffer from breathing or circulatory problems.
- Ethical and moral issues are associated with the destruction of embryos.

Dr David Wells, AgResearch

Cloning allows a rapid spread of valuable livestock into commercial use. It also allows the livestock industry to respond rapidly to market changes in the demand for certain traits in livestock products (e.g. lean meat). Photo above: Ten healthy clones were produced from a single cow (differences in coat colour patterns arise from the random migration of pigment cells in early embryonic development).

Cloning and conservation



Dr David Wells, AgResearch

Enderby Island is part of the Auckland Islands group, south of New Zealand. A distinct cattle breed arose there after 90 years of isolation, following their abandonment on the island in the early 1900s. In attempts to restore the island ecology, most of the cattle were destroyed, but semen and egg cells were taken and stored. In 1992, it was discovered that two cattle remained on the island, a cow (Lady) and her calf (which later died). Lady produced a bull calf by *in vitro* fertilisation and implantation in a surrogate mother and Lady herself was cloned using SCNT. Two surviving clones were bred to the bull calf and the small population is now in its third generation. The Enderby Island cattle remain the only rare breed to be saved from extinction using SCNT.

1. (a) What is SCNT? _____

   (b) How does SCNT differ from embryo splitting? _____

2. What are the benefits of using SCNT to produce livestock with desirable traits? _____

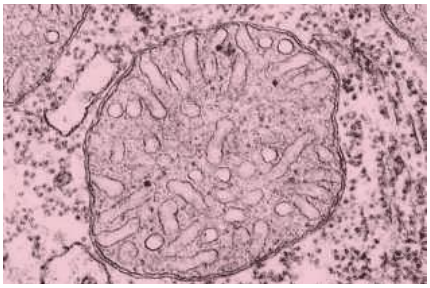
3. Discuss the biological implications of SCNT: _____

ISBN: 978-1-98-856655-9

The effectiveness of somatic cell nuclear transfer

- Although the principle behind the technique is relatively simple, the success rate for viable births is very low. It is still not currently a viable technique for mass production of clones and is limited to specific uses such as in cloning high value livestock, in endangered species recovery, and in medical research.
- Although the clone and the donor have identical nuclear DNA, the mitochondrial DNA is different. This may lead to incompatibilities and may contribute to the low success rate.

Somatic cell nuclear transfer has been used to clone endangered species including the Bucardo (above), the Mouflon, and the Enderby Island cattle in New Zealand.



SCNT does not produce perfect clones. The mitochondrial DNA is from the donor egg cell cytoplasm, and not from the donor of the nuclear DNA. This may introduce some physiological variation in the clones.

Some people have suggested using SCNT to bring back extinct animals e.g. the woolly mammoth. This is highly unlikely for a variety of reasons, including inability to replicate the full DNA sequence.

4. How would cloning be useful for the conservation of animal species? _____

5. Why is SCNT not currently a viable technique for the mass production of clones? _____

6. As a class, discuss the ethics of possibly using SCNT to bring back extinct species. Is there a difference between bringing back species that (may have) died out naturally, e.g. the giant short-faced kangaroo, and species that were deliberately hunted to extinction such as the thylacine, or the dodo?

7. Carry out an internet search on the effectiveness of SCNT. What species have been cloned by this technique? How many embryos actually survived to birth, and how many survived to adulthood? What is the main usage of SCNT in the scientific community. Have there been any developments that have made SCNT more viable over time?

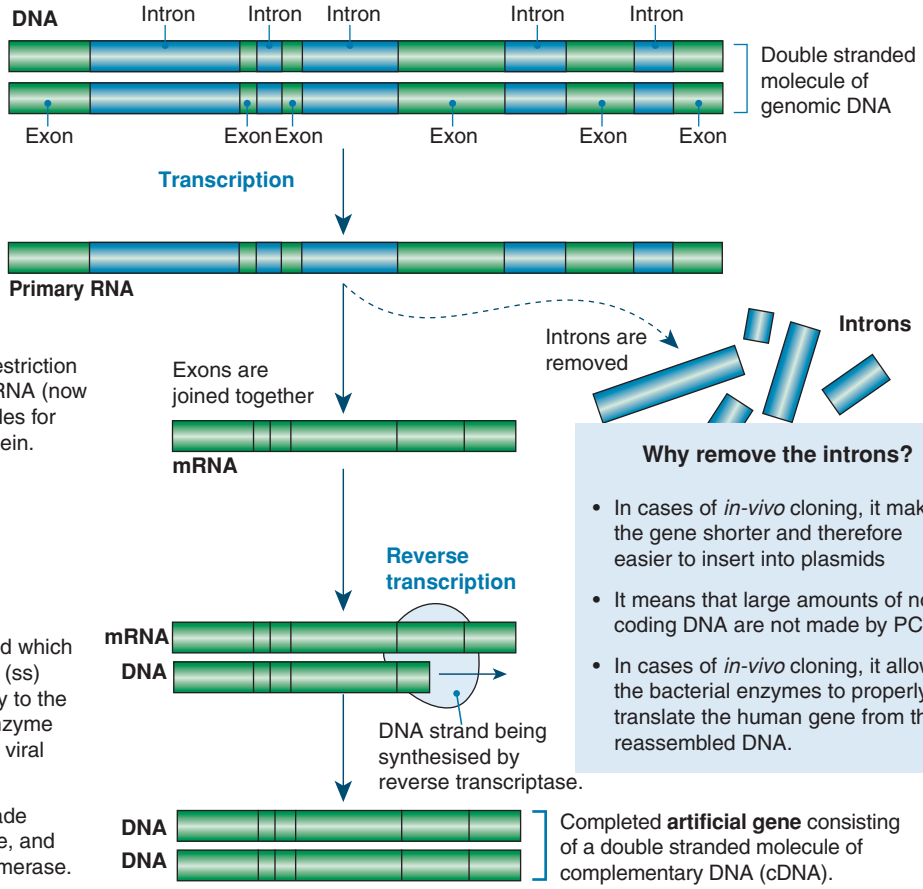
ISBN: 978-1-98-856655-9

136 Preparing a Gene for Cloning

Key Idea: Eukaryotic genes contain introns, which must be removed before a gene can be inserted into a prokaryotic cell for *in vivo* cloning.

The presence of introns (sequences of a gene's DNA that do not code for proteins) presents a problem when preparing a eukaryotic gene for insertion into a prokaryotic cell. Prokaryotes are the most commonly used organism in large scale culture of gene products but do not distinguish between introns and exons (coding sequences). The solution is to engineer a eukaryotic gene that can be transcribed and translated (expressed) by a prokaryote. This is achieved using the retroviral enzyme **reverse transcriptase**, which copies the mature mRNA (containing exons only) to produce a complementary strand of DNA. This task is important in both *in vivo* gene cloning and *ex vivo* gene cloning (by PCR) because it creates a gene ready for amplification.

1. Double stranded DNA of a gene from a eukaryotic organism (e.g. human) containing introns.
2. Transcription creates a **primary RNA** molecule as a part of the cell's normal gene expression.
3. The introns are removed by restriction enzymes to form a mature mRNA (now excluding the introns) that codes for the production of a single protein.
4. The mRNA is extracted from the cell and purified.
5. Reverse transcriptase is added which synthesises a single stranded (ss) DNA molecule complementary to the mRNA. In retroviruses, this enzyme makes a DNA strand from the viral ssRNA.
6. The second DNA strand is made by using the first as a template, and adding the enzyme DNA polymerase.



Why remove the introns?

- In cases of *in-vivo* cloning, it makes the gene shorter and therefore easier to insert into plasmids
- It means that large amounts of non-coding DNA are not made by PCR.
- In cases of *in-vivo* cloning, it allows the bacterial enzymes to properly translate the human gene from the reassembled DNA.

1. What is the role of restriction enzymes in preparing a clone? _____

2. (a) Why are introns removed before cloning a gene? _____

   (b) What is the role of reverse transcriptase in this process? _____

3. What is the normal role of reverse transcriptase? _____

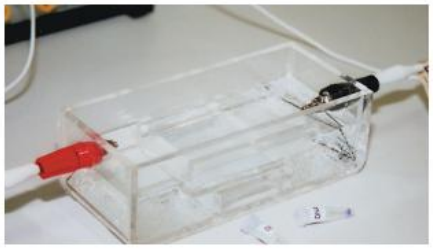
ISBN: 978-1-98-856655-9

137 Making Recombinant DNA

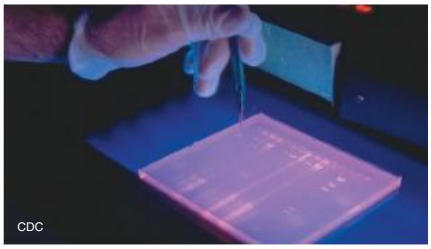
Key Idea: Recombinant DNA (rDNA) is produced by first isolating (or synthesising) a DNA sequence, then inserting it into the genome of a different organism, e.g. a bacterium.
Recombinant DNA (rDNA) is produced by combining genetic material from two or more different sources. The production of rDNA is possible because the DNA of every organism is made of the same building blocks (nucleotides). rDNA allows a gene from one organism to be moved into, and expressed in, a different organism. Two important tools are used to create rDNA. **Endonucleases** (such as restriction enzymes or the CRISPR-Cas9 system) cut the DNA and the enzyme **DNA ligase** is used to join the sections of DNA together.

Overview: How is recombinant DNA made?

The target gene is prepared using mRNA and reverse transcriptase to turn the mRNA into DNA with the introns removed. The gene is then amplified using PCR, producing multiple copies of the gene. Recognition sites for restriction enzymes are added to the gene during PCR.



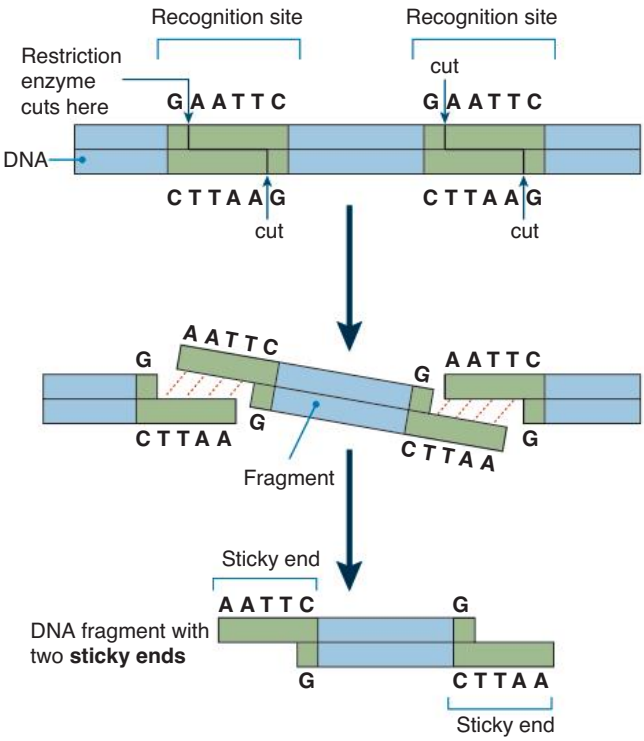
Restriction enzymes are added to cut the gene and the plasmid into which the gene will be spliced. The samples are mixed with ethidium bromide, which fluoresces under UV light, and run on an electrophoresis gel to separate the DNA and plasmid from unwanted material.



After separation, the gel is placed on a UV viewing platform. The areas of the gel containing the gene and plasmid are cut out. The pieces of gel are melted and mixed together along with a ligase enzyme which will join the target gene and the plasmid.

What are restriction enzymes?

- A **restriction enzyme** is an enzyme that cuts a double-stranded DNA molecule at a specific **recognition site** (a specific DNA sequence). There are many different types of restriction enzymes, each with a unique recognition site.
- Some restriction enzymes produce DNA fragments with two **sticky ends** (right). A sticky end has exposed nucleotide bases at each end. DNA cut in such a way is able to be joined to other DNA with matching sticky ends. Such joins are specific to their recognition sites.
- Some restriction enzymes produce a DNA fragment with two blunt ends (ends with no exposed nucleotide bases). The piece from which it is removed is also left with blunt ends. DNA cut in such a way can be joined to any other blunt end fragment. Unlike sticky ends, blunt end joins are non-specific because there are no sticky ends to act as specific recognition sites.



1. What is the purpose of restriction enzymes in making recombinant DNA? _____

_____

_____

2. What is the difference between sticky end and blunt end fragments? _____

_____

_____

3. Why is it useful to have many different kinds of restriction enzymes? _____

_____

_____

ISBN: 978-1-98-856655-9

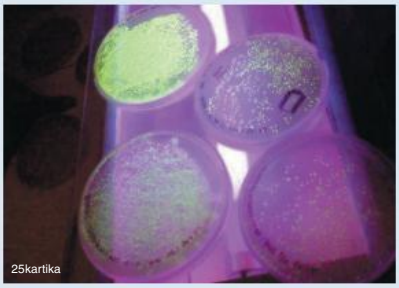
Creating a recombinant DNA plasmid

1. Two pieces of DNA are cut by the same restriction enzyme. They will produce fragments with matching sticky ends.

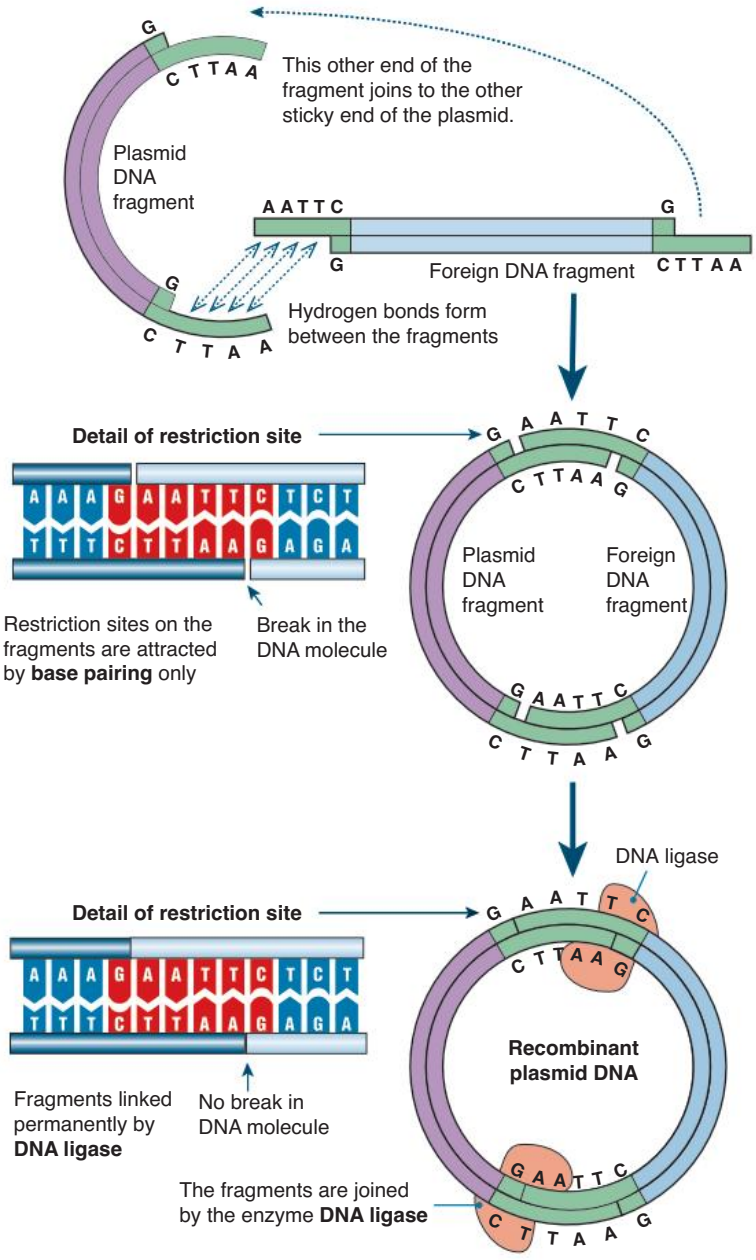
2. Fragments with matching sticky ends can be joined by base-pairing. This process is called **annealing.** This allows DNA fragments from different sources to be joined.

   The DNA fragments are joined by the enzyme **DNA ligase**, which catalyses the formation of a phosphodiester bond. This produces a molecule of recombinant DNA.

3. The joined fragments will usually form either a linear or a circular molecule, as shown here (right) as recombinant plasmid DNA.



pGLO is a plasmid engineered to contain Green Fluorescent Protein (*gfp*). pGLO has been used to create fluorescent organisms, including the bacteria above (bright patches on agar plates).



4. Explain in your own words the two main steps in the process of joining two DNA fragments together:

   (a) Annealing: _____

   _____

   (b) DNA ligase: _____

   _____

5. Explain why ligation can be considered the reverse of the restriction digestion process: _____

   _____

6. Why can recombinant DNA be expressed in any kind of organism, even if it contains DNA from another species?

   _____

   _____

   _____

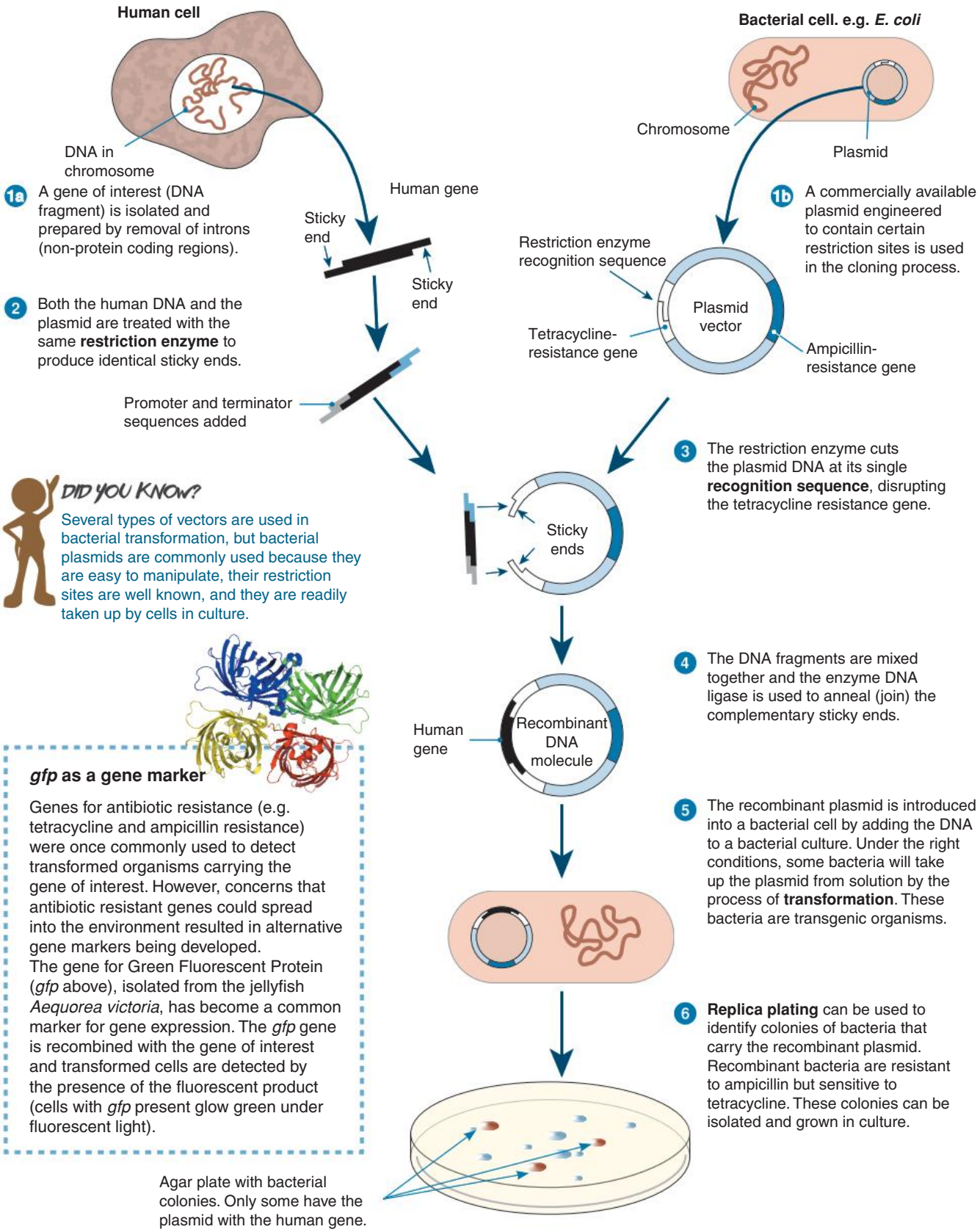
   _____

ISBN: 978-1-98-856655-9

138 Gene Cloning

Key Idea: Recombinant plasmids taken up into a host organism are replicated when the host's DNA is replicated. This produces large quantities of the recombinant DNA.

We have already seen that recombinant DNA techniques can be used to insert a gene into a plasmid. The plasmid can then be used to transmit the gene to another organism (e.g. *E. coli* bacteria). Once inside *E. coli*, the gene is replicated along with the host DNA. This technique is called **gene cloning** and produces multiple copies of the gene. The recombinant plasmid must be able to replicate inside its host, it must have one or more sites at which a restriction enzyme can cut, and it must have some kind of genetic marker that allows them to be identified. **Replica plating** is often used to identify organisms that have produced the gene of interest.



DID YOU KNOW?

Several types of vectors are used in bacterial transformation, but bacterial plasmids are commonly used because they are easy to manipulate, their restriction sites are well known, and they are readily taken up by cells in culture.

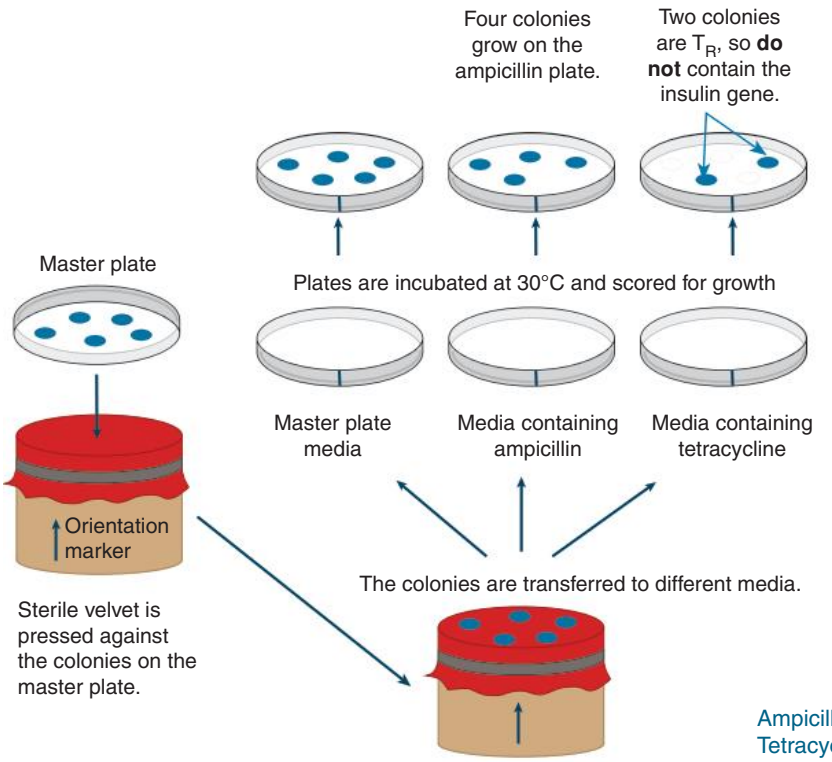
gfp as a gene marker

Genes for antibiotic resistance (e.g. tetracycline and ampicillin resistance) were once commonly used to detect transformed organisms carrying the gene of interest. However, concerns that antibiotic resistant genes could spread into the environment resulted in alternative gene markers being developed.

The gene for Green Fluorescent Protein (*gfp* above), isolated from the jellyfish *Aequorea victoria*, has become a common marker for gene expression. The *gfp* gene is recombined with the gene of interest and transformed cells are detected by the presence of the fluorescent product (cells with *gfp* present glow green under fluorescent light).

ISBN: 978-1-98-856655-9

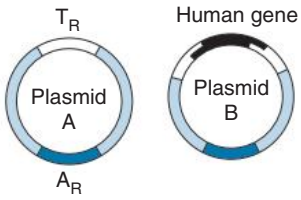
Replica plating identifies colonies with desirable qualities



After gene cloning, it is important to be able to identify the colonies in which transformation has occurred. This is achieved by **replica plating**.

Replica plating transfers colonies from a master plate to test plates enriched with specific nutrients or antibiotics. The original pattern of colonies is maintained during the transfer. Growth (or lack of) on the test plates can be used to identify colonies of interest (e.g. colonies containing the insulin gene).

In the example on the left, colonies are tested for their susceptibility to the antibiotic, tetracycline. Those with ampicillin resistance but no tetracycline resistance contain the insulin gene (plasmid B). The insertion of the insulin gene has interrupted the tetracycline gene, so they are sensitive to tetracycline.



Ampicillin resistance (A_R):	YES	YES
Tetracycline resistance (T_R):	YES	NO

1. What is the purpose of gene cloning? _____

2. Explain how a human gene is removed from a chromosome and placed into a plasmid: _____

3. (a) What is the purpose of replica plating? _____

   (b) In the replica plating example above, explain how the colonies with the recombinant plasmids are identified: _____

   (c) What can you say about the colony that did not grow on the ampicillin plate? _____

4. Why is the *gfp* marker a more desirable gene marker than genes for antibiotic resistance? _____

ISBN: 978-1-98-856655-9

139 Using Recombinant Plasmids in Industry

Key Idea: Inserting useful genes into bacteria to produce biofactories can solve the problem of shortages in the manufacturing and food industries.

Chymosin, also known as rennin, is an enzyme that digests milk proteins. It is the active ingredient in rennet, a substance used by cheesemakers to clot milk into curds. Traditionally, rennin is extracted from 'chyme', which is the stomach secretions of suckling calves, hence its name of chymosin. In the 1960s, a shortage of chymosin was limiting the volume of cheese produced. Enzymes from fungi were used as an alternative but were unsuitable because they caused variations in the cheese flavour. We can now use recombinant DNA technology to produce industrially important enzymes in large quantities. Rennet is used as a coagulant in some yogurts and in many other processed foods, where milk is used in a coagulated form

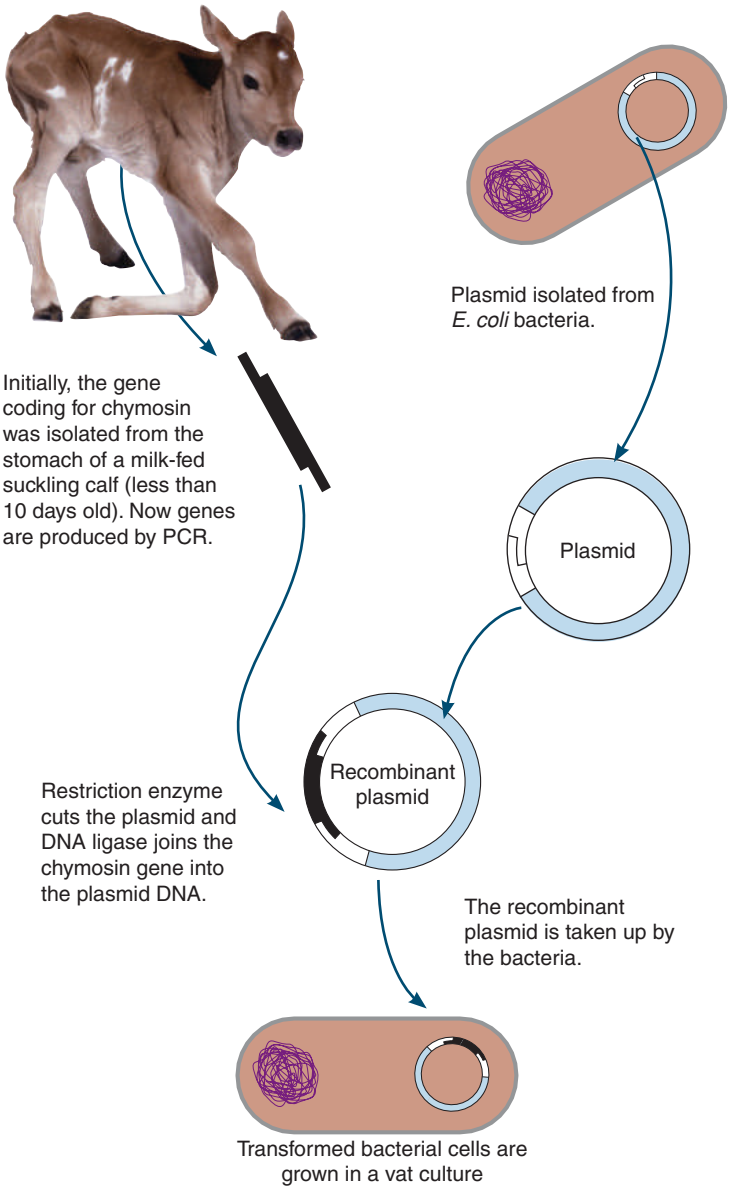
Concept 1
Enzymes are proteins made up of amino acids. The amino acid sequence of chymosin can be determined and the mRNA coding sequence for its translation identified.

Concept 2
Reverse transcriptase can be used to synthesise a DNA strand from the mRNA. This process produces DNA without the introns, which cannot be processed by bacteria.

Concept 3
DNA can be cut at specific sites using **restriction enzymes** and rejoined using **DNA ligase.** New genes can be inserted into self-replicating bacterial **plasmids**.

Concept 4
Under certain conditions, bacteria are able to lose or take up plasmids from their environment. Bacteria are readily grown in vat cultures at little expense.

Concept 5
The protein is made by the bacteria in large quantities.



Techniques

- The amino acid sequence of chymosin is first determined and the RNA codons for each amino acid identified.
- mRNA matching the identified sequence is isolated from the stomach of young calves. **Reverse transcriptase** is used to transcribe mRNA into DNA. The DNA sequence can also be made synthetically once the sequence is determined.
- The DNA is amplified using PCR.
- Plasmids from *E. coli* bacteria are isolated and cut using **restriction enzymes.** The DNA sequence for chymosin is inserted using **DNA ligase**.
- Plasmids are returned to *E. coli* by placing the bacteria under conditions that induce them to take up plasmids.

Outcomes

The transformed bacteria are grown in vat culture. Chymosin is produced by *E. coli* in packets within the cell that are separated during the processing and refining stage.

Recombinant chymosin entered the marketplace in 1990. It established a significant market share because cheesemakers found it to be cost effective, of high quality, and in consistent supply. Most cheese is now produced using recombinant chymosin such as CHY-MAX.

Further applications

A large amount of processing is required to extract chymosin from *E.coli.* There are now a number of alternative bacteria and fungi that have been engineered to produce the enzyme. Most chymosin is now produced using the fungi ***Aspergillus niger*** and ***Kluyveromyces lactis***. Both are produced in a similar way as that described for *E. coli*.

ISBN: 978-1-98-856655-9

Enzymes from GMOs are widely used in the baking industry. Maltogenic α-amylase from *Bacillus subtilis* bacteria is used as an anti-staling agent to prolong shelf life. Hemicellulases from *B. subtilis* and xylanase from the fungus *Aspergillus oryzae* are used to improve dough, crumb structure, and volume during the baking process.

Romain Behar

Lipase from *Aspergillus oryzae* is used to process palm oil to produce substitutes for cocoa butter (above), which have similar textural qualities but lower cost.

Dual Freq

Acetolactate decarboxylase from *B. subtilis* is an enzyme used in the brewing industry. It reduces maturation time of the beer by bypassing a rate-limiting step.

1. Describe the main use of chymosin: _____

2. What was the traditional source of chymosin? _____

3. Summarise the key concepts that allowed the development of the industrial process for producing chymosin:

4. Discuss how the gene for chymosin was isolated and how the technique could be applied to isolating other genes:

5. Describe three advantages of using chymosin produced by GM bacteria over chymosin from traditional sources:

   (a) _____

   (b) _____

   (c) _____

6. Explain why the fungus *Aspergillus niger* is now more commonly used to produce chymosin instead of *E. coli*:

ISBN: 978-1-98-856655-9

140 Engineering for Improved Nutrition

Key Idea: The use of recombinant DNA to build a new metabolic pathway has greatly increased the nutritional value of a variety of rice.

Beta-carotene (β-carotene) is a precursor to **vitamin A** which is involved in many functions including vision, immunity, fetal development, and skin health. Vitamin A deficiency is common in developing countries, where up to 500,000 children suffer from night blindness, and death rates due to infections are high due to a lowered immune response. Providing enough food containing useful quantities of β-carotene is difficult and expensive in many countries. Golden rice was developed by scientists to help overcome the problem of vitamin A deficiency.

Concept 1

Rice is a staple food in many developing countries. It is grown in large quantities and is available to most of the population, but it lacks many of the essential nutrients required by the human body for healthy development. It is low in β-carotene.

Concept 2

Rice plants produce β-carotene but not in the edible rice endosperm. Engineering a new biosynthetic pathway would allow β-carotene to be produced in the endosperm. Genes expressing enzymes for carotene synthesis can be inserted into the rice genome.

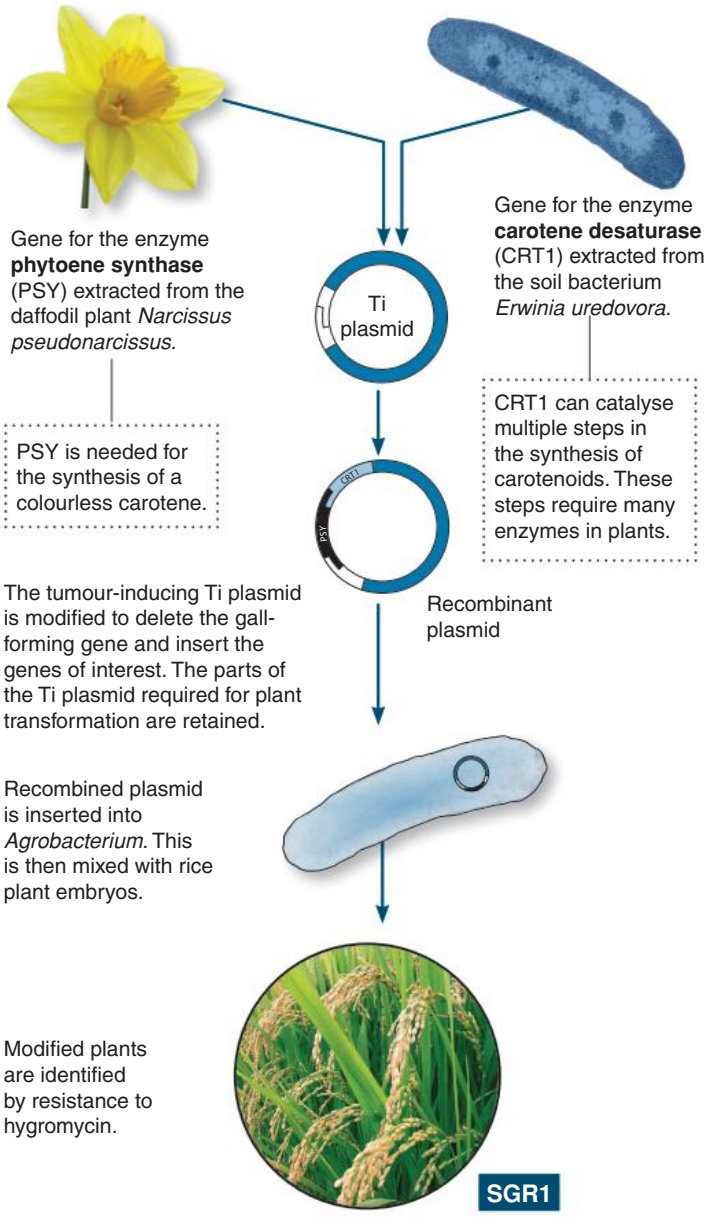
Concept 3

The enzyme, **carotene desaturase** (CRT1) in the soil bacterium *Erwinia uredovora*, catalyzes multiple steps in carotenoid biosynthesis. **Phytoene synthase** (PSY) overexpresses a colourless carotene in the daffodil plant *Narcissus pseudonarcissus*.

Concept 4

DNA can be inserted into an organism's genome using a suitable vector. *Agrobacterium tumefaciens* is a gall-forming bacterial plant pathogen that is commonly used to insert novel DNA into plants.

The development of golden rice



Techniques

- The **PSY** gene from daffodils and the **CRT1** gene from *Erwinia uredovora* are sequenced.
- DNA sequences are synthesised into packages containing the CRT1 or PSY gene, terminator sequences, and **endosperm specific promoters** (these ensure expression of the gene only in the edible portion of the rice).
- The ***Ti* plasmid** from *Agrobacterium* is modified using restriction enzymes and DNA ligase to delete the gall-forming gene and insert the synthesised DNA packages. A gene for resistance to the antibiotic **hygromycin** is also inserted so that transformed plants can be identified later. The parts of the *Ti* plasmid required for plant transformation are retained.
- Modified *Ti* plasmid is inserted into the bacterium.
- *Agrobacterium* is incubated with rice plant embryo. Transformed embryos are identified by their resistance to hygromycin.

Outcomes

The rice produced had endosperm with a distinctive yellow colour. Under greenhouse conditions golden rice (**SGR1**) contained 1.6 µg per g of carotenoids. Levels up to five times higher were produced in the field, probably due to improved growing conditions.

Further applications

Further research on the action of the PSY gene identified more efficient methods for the production of β-carotene. The second generation of golden rice now contains up to 37 µg per g of carotenoids. Golden rice was the first instance where a complete biosynthetic pathway was engineered. The procedures could be applied to other food plants to increase their nutrient levels.

ISBN: 978-1-98-856655-9

Golden rice: A controversial solution

IRRI

Golden rice (top bowl) is promoted as one way to increase the beta-carotene (and thus vitamin A) intake in countries where rice is a staple part of the diet. However, there are many groups that actively resist its promotion and use (e.g. Greenpeace).

Many of these anti-GM groups believe there is no need for golden rice because people can obtain enough beta-carotene by eating a variety of fruits and vegetables. They are also worried about the possibility of golden rice contaminating ordinary rice crops.

In June 2016 the The National Academies of Sciences, Engineering, and Medicine released the results of an extensive study, reporting there was no evidence to suggest GM crops were unsafe and that GM crops are as safe to eat as non-GM crops.

1. Describe the basic methodology used to create golden rice: _____

2. Explain how scientists ensured β-carotene was produced in the endosperm: _____

3. What property of *Agrobacterium tumefaciens* makes it an ideal vector for introducing new genes into plants?

4. (a) How could this new variety of rice reduce disease in developing countries? _____

   (b) Absorption of vitamin A requires sufficient dietary fat. Explain how this could be problematic for the targeted use of golden rice in developing countries:

5. Explain why golden rice is a controversial product: _____

ISBN: 978-1-98-856655-9

141 Using Recombinant Plasmids in Medicine

Key Idea: By using microorganisms to make human insulin, problematic issues of cost, allergic reactions, and ethics have been addressed.

Type I diabetes mellitus is a metabolic disease caused by a lack of **insulin**. Around 25 people in every 100,000 suffer from type I diabetes and it is treatable only with injections of insulin. In the past, insulin was taken from the pancreatic tissue of cows and pigs and purified for human use. The method was expensive and some patients had severe allergic reactions to the foreign insulin or its contaminants. Now, we can use biotechnology to produce insulin in large quantities, inexpensively, to help people with diabetes.

Concept 1

DNA can be cut at specific sites using **restriction enzymes** and joined together using **DNA ligase**. Genes can be inserted into self-replicating bacterial **plasmids** at the point where the cuts are made.

Concept 2

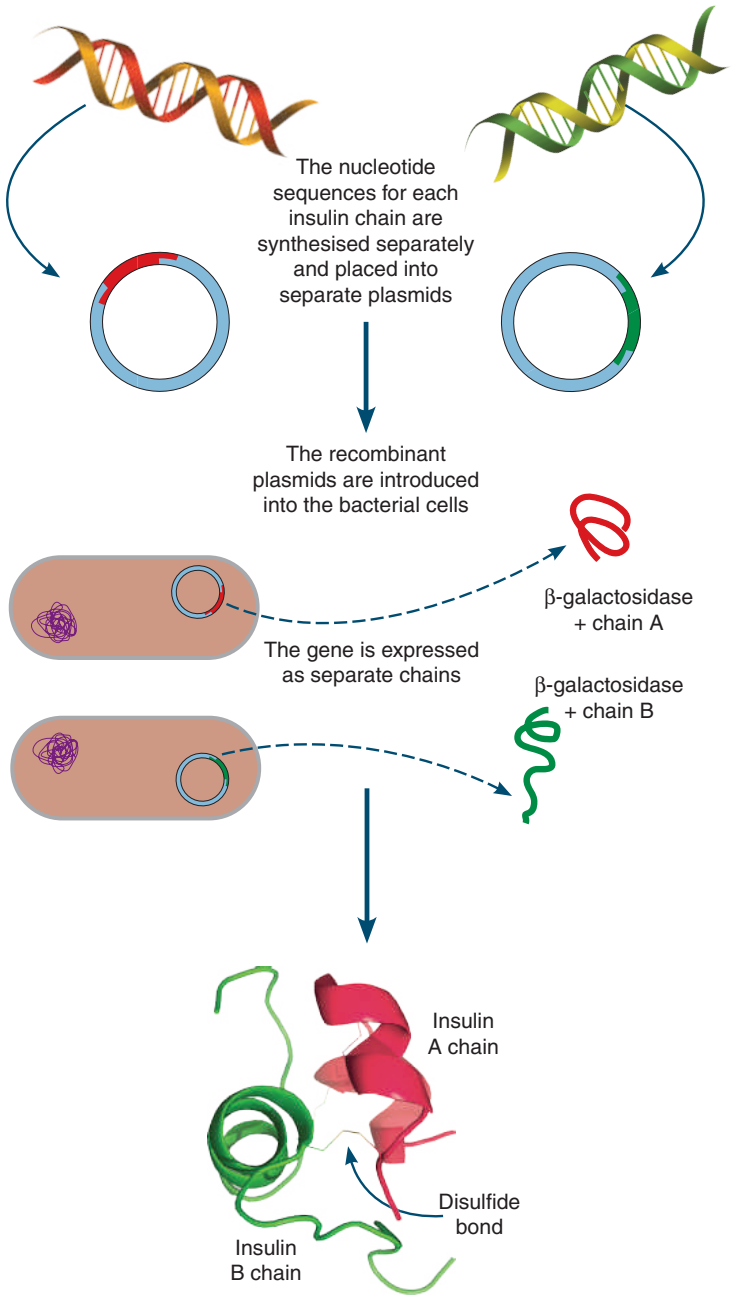
Plasmids are small, circular pieces of DNA found in some bacteria. They usually carry genes useful to the bacterium. *E. coli* plasmids can carry promoters required for the transcription of genes.

Concept 3

Under certain conditions, Bacteria are able to lose or pick up plasmids from their environment. Bacteria can be readily grown in vat cultures at little expense.

Concept 4

The DNA sequences coding for the production of the two polypeptide chains (A and B) that form human insulin can be isolated from the human genome.



Techniques

- The gene is chemically synthesised as two DNA sequences, one for the insulin A chain and one for the insulin B chain. The two sequences are small enough to be inserted into a plasmid.
- Plasmids are extracted from *Escherichia coli*. The gene for the bacterial enzyme β-galactosidase is located on the plasmid. To make the bacteria produce insulin, the insulin gene must be linked to the β-galactosidase gene, which carries a promoter for transcription.
- Restriction enzymes are used to cut plasmids at the appropriate site and the A and B insulin sequences are inserted. The sequences are joined with the plasmid DNA using DNA ligase.
- The recombinant plasmids are inserted back into the bacteria by placing them together in a culture that favours plasmid uptake by bacteria.
- The **transgenic bacteria** are then grown and multiplied in vats under carefully controlled growth conditions.

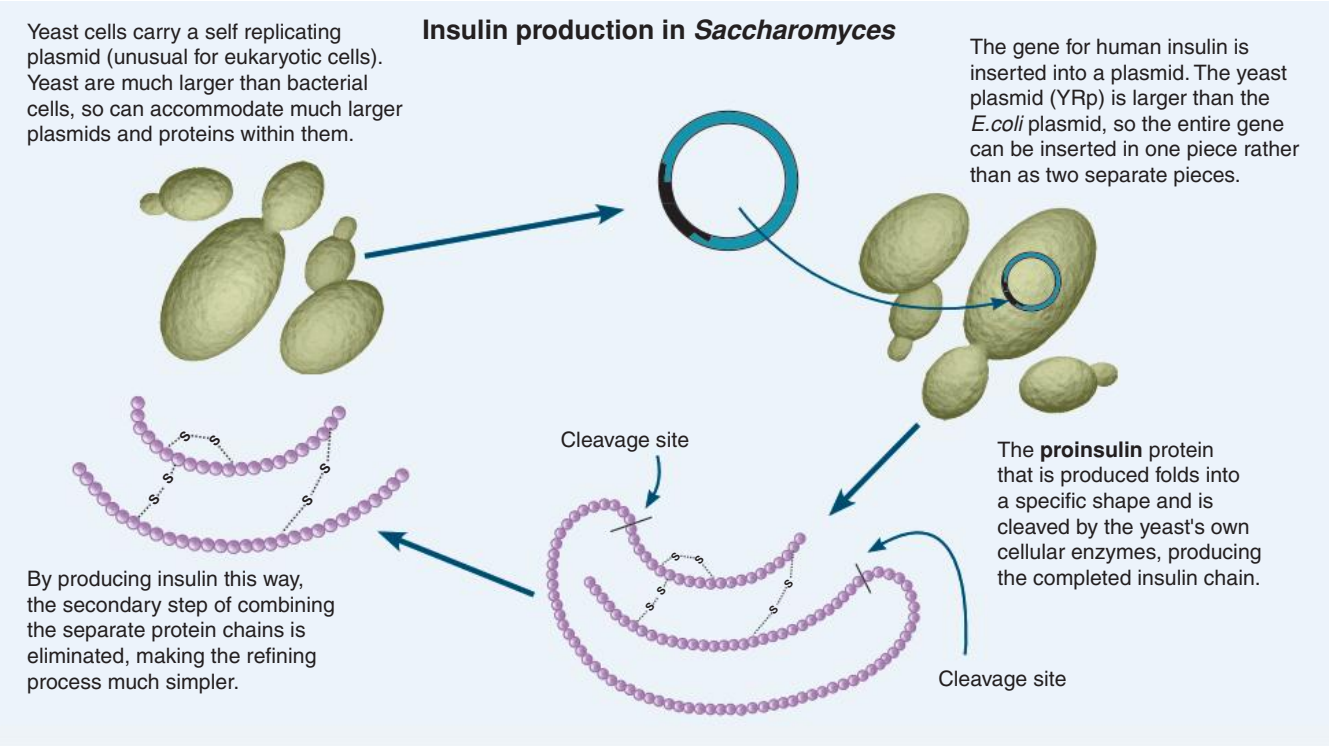
Outcomes

The product consists partly of β-galactosidase, joined with either the A or B chain of insulin. The chains are extracted, purified, and mixed together. The A and B insulin chains connect via disulfide cross linkages to form the functional insulin protein. The insulin can then be made ready for injection in various formulations.

Further Applications

The techniques used to produce human insulin from genetically modified bacteria can be applied to a range of human proteins and hormones. Proteins currently being produced include human growth hormone, interferon, and factor VIII.

ISBN: 978-1-98-856655-9



1. Describe the three major problems associated with the traditional method of obtaining insulin to treat diabetes:

   (a) _____

   (b) _____

   (c) _____

2. Explain the reasoning behind using *E. coli* to produce insulin and the benefits that GM technology has brought to diabetics:

   _____

   _____

   _____

3. Explain why, when using *E. coli*, the insulin gene is synthesised as two separate A and B chain nucleotide sequences:

   _____

   _____

4. Why are the synthetic nucleotide sequences (‘genes’) 'tied' to the β-galactosidase gene? _____

   _____

   _____

5. Yeast (*Saccharomyces cerevisiae*) is also used in the production of human insulin. Discuss the differences in the production of insulin using yeast and *E. coli* with respect to:

   (a) Insertion of the gene into the plasmid: _____

   _____

   _____

   (b) Secretion and purification of the protein product: _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

142 Engineering for Insect Resistance

Key Idea: Up to one fifth of the world's crops are lost due to insects each year. Losses can be reduced through the use of genetic engineering to introduce the Bt gene into crop plants.

A key goal in horticulture is the reduction of insect crop damage. Normally, this is done using sprays. However, this requires a lot of effort and leaves potentially harmful chemical residues on the food and in the environment. Using genetic engineering to produce crop plants with their own in-built insect deterrents can result in greater crop yields and reduced chemical use.

Bt toxin

Bacillus thuringiensis is a soil bacterium. It also occurs naturally in the gut of caterpillars and on leaf surfaces. The bacteria form spores that are associated with crystalline proteins called δ-endotoxins. These are lethal to butterfly and moth larvae but do not affect other insects such as beetles or bees (or any other animal). For this reason, the Bt toxin has been used as a targeted insecticide since the 1960s.

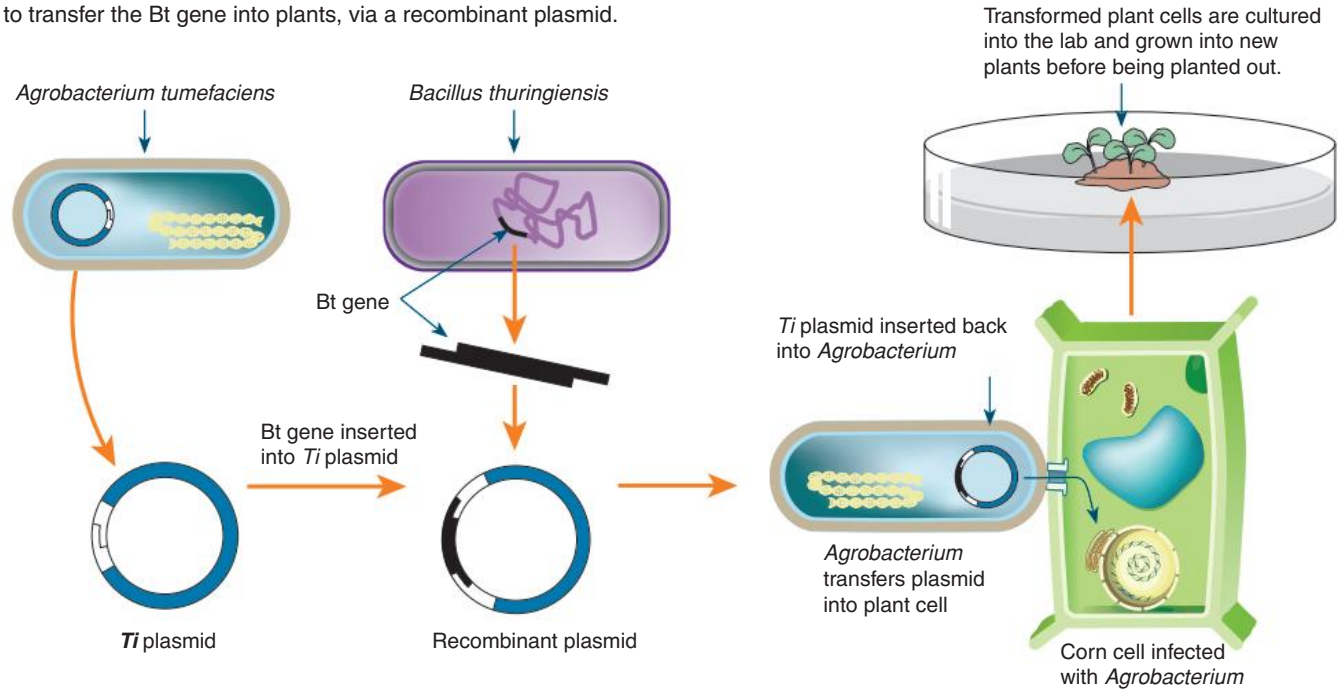
In 1996 the seed company Monsanto released its first version of Bt corn. This corn had been genetically modified to contain the gene that produces the Bt protein. The target insect pest for Bt corn is the larval stage of the European corn borer, which causes hundreds of millions of dollars worth of damage to crops annually.

USDA

The effects of the Bt toxin on insect deterrence. The plant on the right has been treated with Bt toxin before being exposed to caterpillars. The plant on the left had not been treated with Bt toxin.

Producing a Bt plant

Genetic engineering has been used to produce **transgenic cotton**, corn, and potato varieties that produce the Bt toxin. The bacterium *Agrobacterium tumefaciens* is commonly used to transfer the Bt gene into plants, via a recombinant plasmid.



1. Name the bacteria that produces Bt toxin: _____

2. Why is Bt toxin a useful insecticide? _____

3. What is the primary target of the Bt toxin in Bt corn? _____

4. Explain how Bt corn is produced using *Agrobacterium tumefaciens*: _____

ISBN: 978-1-98-856655-9

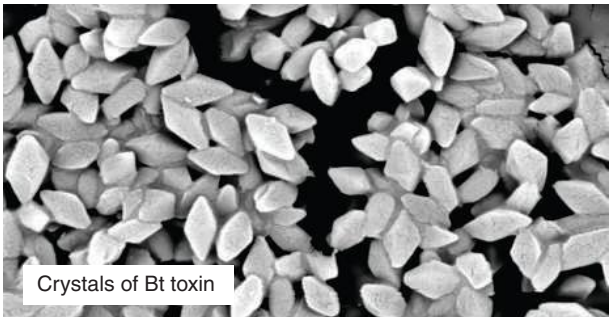
What's killing the monarchs? It's not the corn

Bt corn was developed by the company Monsanto and sales began in 1996. There are many different types of Bt corn, each one engineered to produce the toxin in slightly different ways. One of the first produced was Bt 176.

By 1999 monarch butterfly populations in the American Midwest began declining. During that year, Cornell University published a paper showing that the Bt toxin could be transferred to other plants by the corn's pollen. Pollen landing on milkweed near corn crops could potentially kill the monarch caterpillars that fed exclusively on the milkweed. This resulted in a backlash against Bt corn by environmental activists. However, in 2001 a study was released that argued the toxin in pollen was not causing monarch decline. The toxicity in pollen was due mainly to the Bt 176 variety which was used in less than 2% of the corn grown and was in the process of being phased out. Other Bt corn varieties did not develop enough toxin, or their pollen density was too low to affect monarch caterpillars.

It now appears that there is a related but quite different reason for the Monarch butterfly decline. In 1996, Monsanto also began selling 'Roundup Ready' corn, engineered to withstand glyphosate herbicide. Corn crops could be sprayed with herbicide and, while the weeds die, the corn would keep on growing, allowing less targeted spraying applications. As a result, milkweed which often grew in or near corn crops, was also killed, leaving no food for monarch caterpillars.

So... What's killing the monarchs?

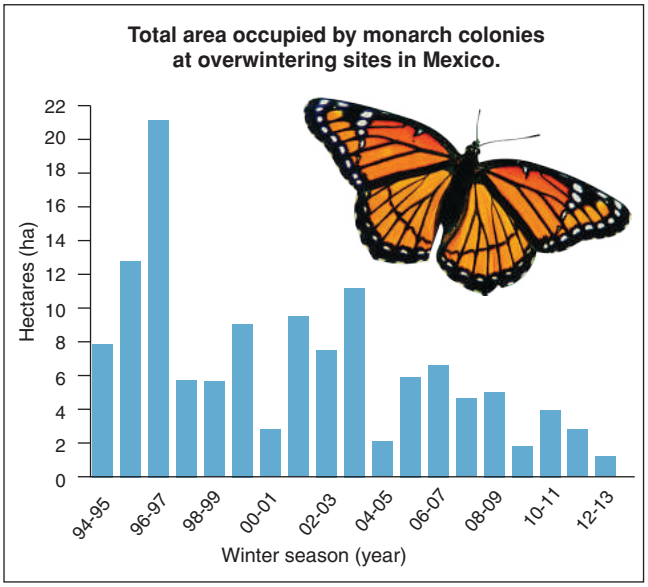


Crystals of Bt toxin

David R. Tribble

Scott McDougall

Above: North American populations of monarchs migrate (above) to overwintering sites in Mexico and California.
Right: Monarch caterpillars feed exclusively on milkweed.



5. As a group, discuss the ethical issues surrounding GM corn and monarch declines. Who is to blame for the decline of monarchs and what can be done to help the population recover? Summarise the main points of your discussion below:

ISBN: 978-1-98-856655-9

143 Agricultural Biotechnology and Biodiversity

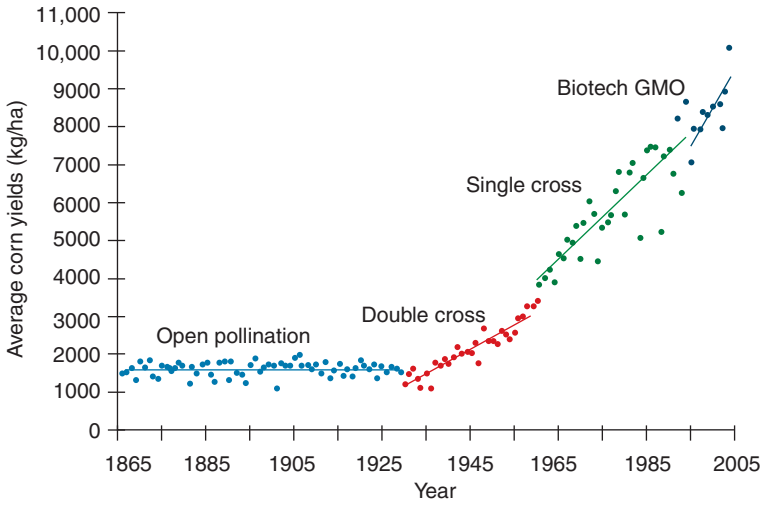
Key Idea: Diversity in agriculture has declined over time due to artificial breeding and the effects of biotechnology.

Historically, agricultural crops used openly pollination. This resulted in a large amount of biodiversity in the crop but low crop yields. Modern crops consist of just a few strains specifically bred to produce high crop yields. Agricultural land today takes up 50% of all habitable land and high yield crops reduce the need to use more land. However, land today is still being converted for agricultural use and, importantly, much of this land is taken from tropical rainforests which are extremely biodiverse. The reasons for this include the simple need to feed the world's population. A continuing aim of science is to continually increase animal and crop yields and reduce the need to use new land, and so help to retain biodiversity.

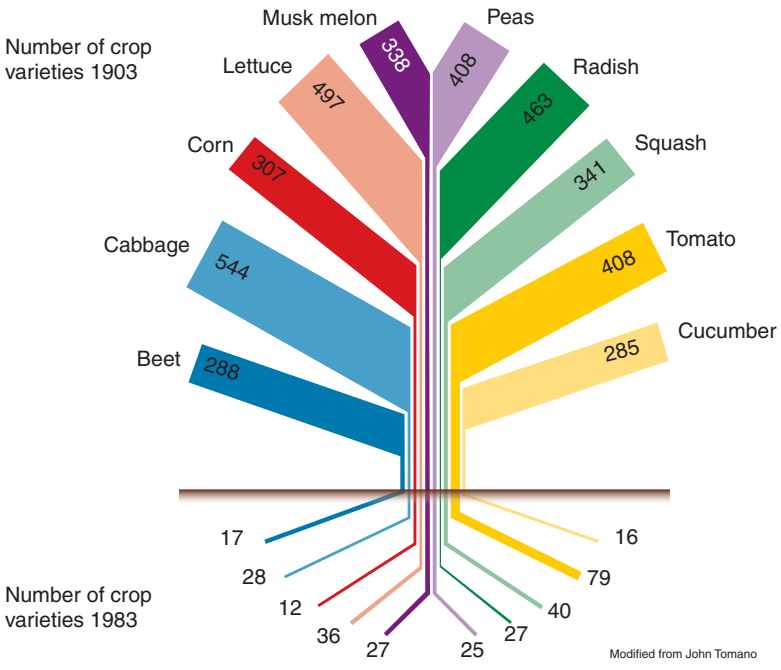
Diversity and biotech

- Before the 1930s corn was selected based on open pollination. Plants producing quality seed were saved for planting the next season. Despite this, crop yield remained relatively static. In the 1920s selection changed from focussing on plants with greater seed mass or ear quality to focussing on disease resistance, high germination rate, and against male plants that appeared barren. This began the trend of increasing crop production.
- Double-cross hybrids were the start of hybrid use. They required four separate strains of parent plants, crossed AxB and CxD, then crossed ABxCD (i.e. a double cross). This was because highly inbred parent strains were not yet developed.
- Single crosses of inbred parent lines became more common from about the 1960s.

Corn yield and breeding technology



The biodiversity of agriculture



- There are an estimated 30,000 species of edible plant in the world. However, only about 150 of these species are cultivated by humans.
- Of those, the vast majority of crop production uses only 30 species of plant, with just 12 producing three quarters of the world's food. Many of these have different varieties but these are essentially variations of the same gene pool.
- Similarly, 97% of meat comes from just 5 groups of animals: chickens, pigs, cattle, sheep, and goats.

1. Why did open pollination produce static crop yields until the 1930s? _____

_____

_____

2. Why has there been such a reduction in crop diversity over time? _____

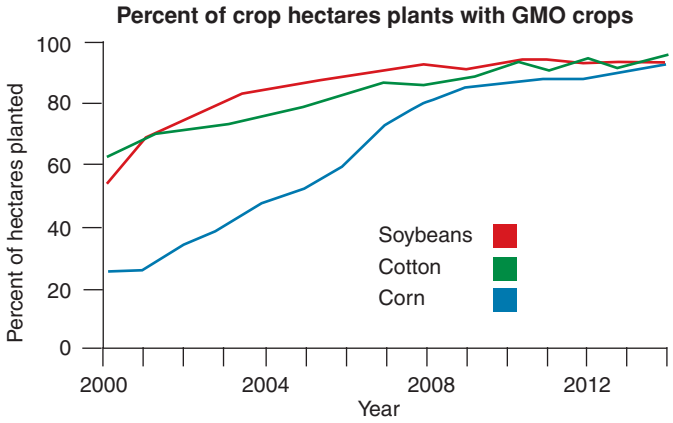
_____

_____

ISBN: 978-1-98-856655-9

Can biotechnology increase biodiversity?

- The very nature of artificial selection reduces biodiversity in the species experiencing the selection. If a selectively bred crop produces a vastly greater yield than other crop varieties it is likely that the majority of growers will want to grow it. This reduces the varieties of crops grown, and so reduces biodiversity further.
- Genetic engineering has the potential to increase biodiversity by introducing new genes into crop species. But again, this can result in the reduction of crop varieties, as growers use only the new modified variety.
- In the United States, GMO crops have virtually replaced all other crop varieties in soybeans, corn, and cotton.



3. Evaluate the effect of biotechnology on biodiversity. Use the information in this activity plus your own research to evaluate the effect of modern breeding techniques and genetic modification on the biodiversity of a named plant crop and the ecosystem (animals that might eat the crop, etc.) associated with it. Useful weblinks can be found on the **BIOZONE Resource Hub.**

   - Name the plant crop
   - Identify at least two sources of information on the named plant and justify why they are reliable sources
   - Identify any major commercial varieties used globally
   - Identify how modern biotechnology has been applied to the crop
   - Discuss the impact of modern biotechnology on both crop biodiversity and the wider ecosystem biodiversity. How might the long term viability of plant crops and the wider ecosystem be affected?

Plant investigated	
Identify at least two sources of information on the named plant and justify why they are reliable sources	
Identify any major commercial varieties used globally	
Identify how modern biotechnology has been applied to the crop	
Discuss the impact of modern biotechnology on both crop biodiversity and the wider ecosystem biodiversity. How might the long term viability of plant crops and the wider ecosystem be affected?	

ISBN: 978-1-98-856655-9

144 Influences on Genetic Technologies

Key Idea: Genetic technologies are influenced by societal, economic, and cultural concerns. There are potential benefits, risks, and ethical issues associated with using genetic technologies.

The use of biotechnologies, including genetic technologies, has many potential benefits in terms of production efficiencies and reduced costs, but their use raises a number of concerns. Some of these include risk to human health, animal welfare issues, and environmental safety. Whenever using these technologies, society has to weigh the benefits against the costs to decide what is best.

Potential benefits of genetic technologies

1. Increase in crop yields, including crops with more nutritional value and with a longer storage life.
2. Decreased use of pesticides, herbicides and animal drug treatments, including antibiotics.
3. Production of crops that tolerate a range of conditions (e.g. drought).
4. Improvement in the health of the human population and the medicines used to achieve it.
5. Development of animal factories for the production of proteins used in manufacturing, the food industry, and health.

Potential risks of genetic technologies

1. Possible loss of biodiversity in crops and livestock.
2. Concerns that the release of GMOs into the environment may be irreversible.
3. Animal welfare and ethical issues: Animals bred to extremes or GM animals may suffer poor health and reduced life span.
4. Possible emergence of pest, insect, or microbial resistance to traditional control methods.
5. May create a monopoly and dependence of developing countries on companies who are seeking to control the world's commercial seed supply.

Issues and solutions of genetic technologies

Issue: The accidental release of genetically modified organisms (GMOs) into the environment.
Problem: Recombinant DNA may be taken up by non-target organisms. These have the potential to become pests or cause disease.
Solution: Rigorous controls on the production and release of GMOs. GMOs could have specific genes deleted so that their growth requirements are met only in controlled environments.

Issue: Loss of biodiversity through use of limited animal breeds or plant varieties.
Problem: The loss of biodiversity reduces the ability of breeders and growers to select for new traits in crops, as needs change

Solution: A wide variety of crops should be used. New genetic lineages could be introduced at regular intervals to ensure diversity. Older varieties should be stored in seed banks.

Issue: Targeted use of transgenic organisms in the environment.
Problem: Once their desired function, e.g. environmental clean-up, is completed, they may be undesirable invaders in the ecosystem.
Solution: GMOs can be engineered to contain 'suicide genes' or metabolic deficiencies so that they do not survive for long in the new environment after completion of their task.

1. Select one of each of the benefits or risks of genetic technologies above, and explain why its is a benefit or risk, and discuss whether one (benefit or cost) outweighs the other.

ISBN: 978-1-98-856655-9

Social influences on biotechnology

- Social influences tend to dictate the ethical uses of biotechnologies: what should it be used for and under what circumstances? Is it acceptable to genetically modify plants or animals, or humans?
- The problem with new and complex technologies is that often the majority of people do not understand how they work and how they can be used, which reduces acceptance and use. Like all technologies, biotechnologies can be used to produce harm as well as good and may produce harm even when initially being used for good.
- For example, genetically modified organisms have the potential to solve the problem of growing more food on less land. GMOs could solve food transport problems by lasting longer when harvested and solve nutritional problems by being engineered to increase their nutritional value. However, they also have the potential to be used as a bioweapon and could affect wild species far beyond what we could predict.


Flavr Savr tomatoes were developed to extend shelf-life.


Golden rice was engineered to improve nutrition.


Engineered bioweapons could wreak havoc on the world.

Economic influences on biotechnology

- Developing any kind of technology usually costs a huge amount of money. Government research facilities may carry out certain specific research, or governments may give out funding to private companies based on the merit of their research but this funding is almost invariability limited and will only fund a small amount of a larger project.
- Private companies developing a biotechnology will therefore aim to commercialise their technology as soon as possible to recoup the costs of its development. They will also aim to keep their methods secret for as long as possible, and to gain and retain as many customers as possible to maximise commercial gain.
- Many biotechnologies are dominated by a few large companies. In seed production for example, the top three selling companies in the USA controlled 60% of the total sales in 2018. This has led to concern that a few companies may have control over many people dependent on their product, such as farmers using genetically modified seed. A few dominant companies could theoretically demand any price for their product.
- However, GE crops often produce a greater yield and require less specific farming methods, reducing costs and increasing profits for farmers.

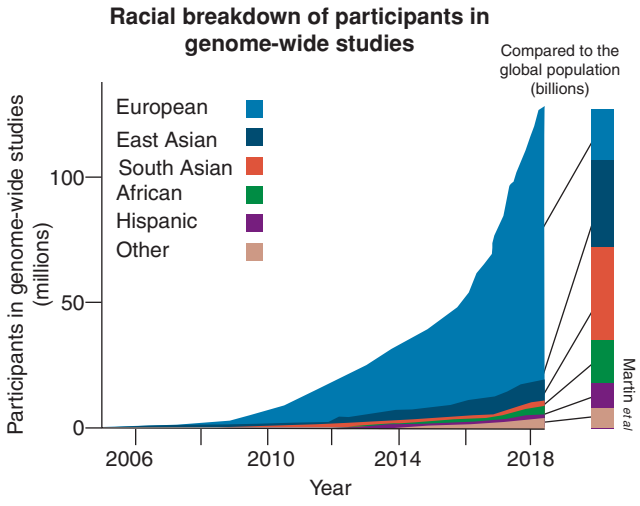

Engineered crops require less accurate spraying.

There are fears only a few seed companies dominate global sales.


Small farm blocks may not be able to afford engineered seeds.

Cultural influences of biotechnology

- Genetic technologies such as DNA profiling, genetic screening, and DNA sequencing have provided a wealth of new information on disease, species relationships, and evolution.
- However, most of the genetic studies on people have focused, often by necessity, on just a few groups of people, usually the majority in a given area. For example if a society is made up of five ethnically diverse groups of people, two of which are by far in the majority, then a genetic study on disease, picking people at random will, by its nature, focus on the two majority groups. Any conclusions from the study will then apply mainly to those two majority groups. If the conclusions about disease in the population are applied in general, then three of the five groups may find their healthcare is compromised.
- Similarly, genetic studies carried out in one country cannot be generally applied to another because the ethnic and genetic composition of the country may be entirely different. Yet this is often what happens, because a study may have only been carried out in a few countries.



ISBN: 978-1-98-856655-9

2. Secondary-sourced investigation report - Influence of social, economic, and cultural contexts on a range of biotechnologies.

- You will gather information including reports, graphs, and tables to access the influence of social economic, and cultural contexts on biotechnology.
- You will focus on the biotechnology involved in one of the following topics: artificial insemination in dairy herd development, genetic modification of humans to prevent disease, cloning pet animals, gene drives to eradicate a pest species. Useful weblinks can be found on the **BIOZONE Resource Hub.**
- Your report will include:
  - An explanation of how the biotechnology is used, in the context you have selected.
  - Assessment of social impacts: e.g. human or animal rights, ethical concerns, stainability.
  - Assessment of economic impacts: e.g. financial costs and benefits for different groups involved.
  - Assessment of cultural impacts: e.g. bias in sampling, religious concerns, effect of cultural beliefs on the uptake of the products produced.
  - Evidence to justify your assessments (e.g. graphs, data, etc from reliable sources (this can be copied and pasted in below).
- Use the tables below to complete your report.

Biotechnology investigated	(a)
Description of how the biotechnology is used	(b)

Assessments of social impacts	Assessments of economic impacts	Assessments of cultural impacts
(c)	(d)	(e)

Evidence for assessment
(f)

ISBN: 978-1-98-856655-9

145 Chapter Review: Did You Get It?

1. Describe each of the following reproductive techniques used in livestock breeding:

   (a) Artificial insemination: _____

   _____

   (b) Embryo transfer: _____

   _____

   (c) *In-vitro* fertilisation: _____

   _____

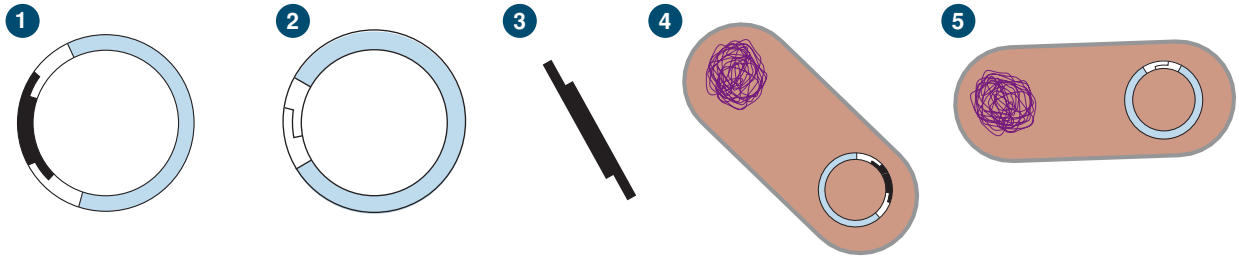
2. A wild flower produces petals with a colour variation from yellow to orange. A grower wants to produce a flower that produces only orange petals. Explain how artificial selection could be used to produce this:

   _____

   _____

   _____

3. Put the numbered items in the correct order for producing a transformed bacterial cell and provide a description of each step in the process:



   _____

   _____

   _____

   _____

   _____

   _____

   _____

4. Explain why crops such as Bt corn that have been engineered to produce their own natural insecticides or repellent have the potential to decrease biodiversity in the wider ecosystem:

   _____

   _____

   _____

   _____

5. Define each of the following and explain when it might be used:

   (a) Restriction enzyme: _____

   _____

   (b) Plasmid: _____

   _____

ISBN: 978-1-98-856655-9

146 Synoptic Assessment: Module 6

1. Two identical twin sisters, Kate and Alison, both inherited one single mutated BRCA1 gene from their mother. This mutation increases the likelihood of developing breast cancer by around 60% prior to the age of 70. Consequently, the twins' mother developed breast cancer at the age of 42, and passed away three years later. Kate, at the age of 37, was diagnosed with the same type of breast cancer as her mother. Two years later, Alison, who was a heavy smoker, developed an unrelated type of lung cancer. Both sisters have one daughter each. Discuss the type and causes of mutations leading to the cancer in both the sisters.

- Explain the difference between germ-line and somatic mutations, linking location of both types of cancer mutation. You may wish to draw a simple tree diagram of fertilisation, twin formation, and cell differentiation to show this.
- Define mutagen, and link to the developed cancers.
- Explain the effect that both cancer mutations will have on the twin sisters' offspring.

Diagram of mutation location

ISBN: 978-1-98-856655-9

2. **The return of the Tasmanian tiger?**
Gene editing technology, using CRISPR, may make this possible one day. University of Melbourne scientists indicate that it could be possible to insert remnants of DNA, obtained from tissue samples of the extinct thylacine (Tasmanian tiger), into the genome of the numbat, an animal that shares 95% of thylacine DNA due to a 'recent' common ancestor, 40 million years ago. The genetically modified 'transgenic' embryo could be implanted into a suitable host such as a dunnart, and successive thylacine embryos could then be cloned. Current technology is currently too time consuming to consider carrying out this procedure, but de-extinction of an apex predator like the thylacine could one day lead to its reintroduction into the Australian ecosystem.

Discuss the processes and consequences of creating a gene edited extinct animal:

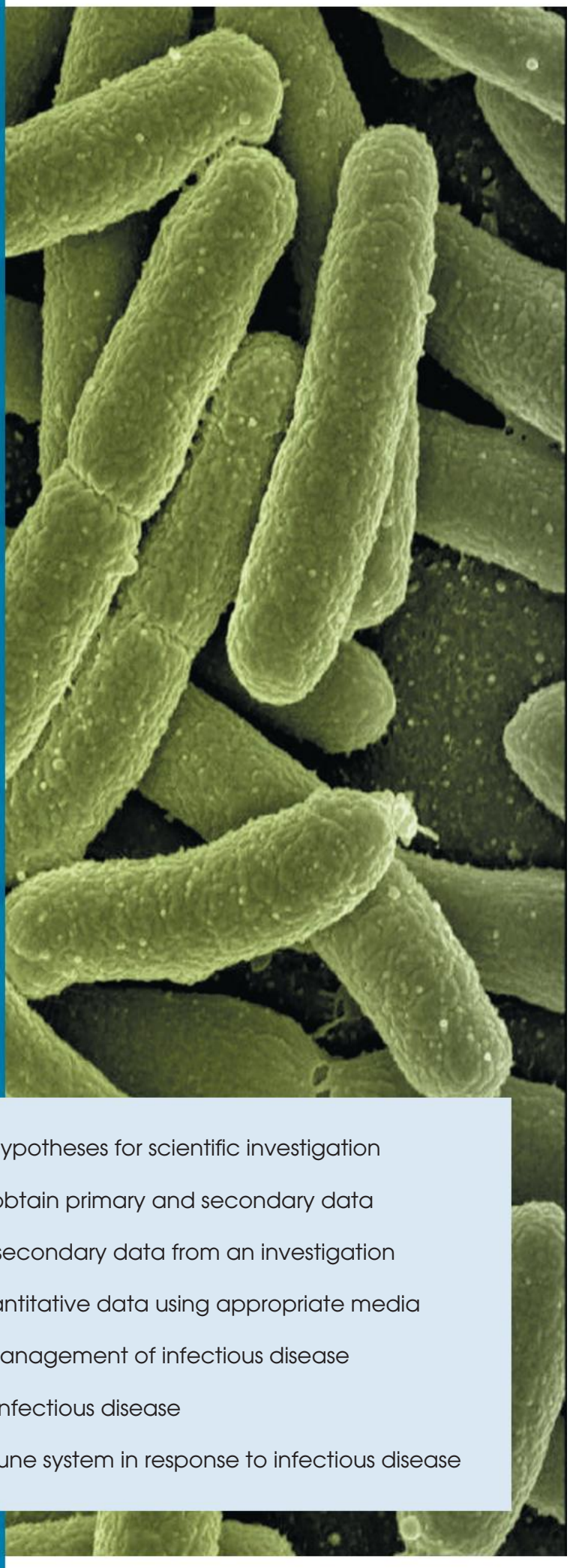
- Define the terms, gene editing and transgenic, and how CRISPR is used as a gene editing tool.
- Compare the differences between gene editing and cloning as genetic technologies.
- Explain any social or ethical considerations that may arise in the de-extinction process of animals.
- Explain what possible effects the genetically edited thylacine might have on an ecosystem's biodiversity if it were to be introduced.

ISBN: 978-1-98-856655-9

MODULE

07

Infectious Disease



Student outcomes:

- Develop and evaluate questions and hypotheses for scientific investigation
- Design and evaluate investigations to obtain primary and secondary data
- Collect valid and reliable primary and secondary data from an investigation
- Select and process qualitative and quantitative data using appropriate media
- Analyse the cause, transmission, and management of infectious disease
- Understand an organism's response to infectious disease
- Understand the role of the human immune system in response to infectious disease

CHAPTER 9

Causes of Infectious Disease

Key terms
bacteria
cellular pathogen
chronic infection
disease complex
disease transmission
epidemic
fungi
infectious disease
Koch's postulates
macroorganism
microorganism
non-cellular pathogen
pandemic
Pasteur
pathogen
prion
protist
vector
virus

Activity number

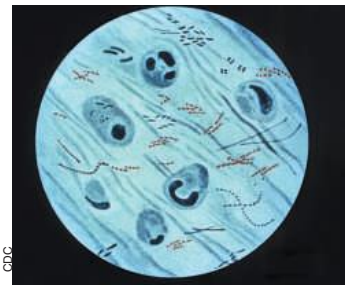
Inquiry question: *How are diseases transmitted?*

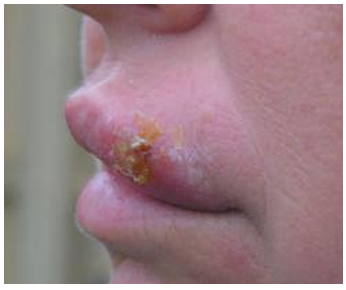
Pathogens cause infectious disease

Key skills and knowledge

- ☐ 1 Understand that pathogens cause infectious disease. Describe how pathogens can be classified as cellular or non-cellular based on whether they are living or non-living entities, respectively. Name the main groups of pathogens (bacteria, fungi, protist, virus, or prion) and classify them as cellular or non-cellular pathogens. **147**
- ☐ 2 Give examples of animal diseases caused by pathogens. For each example, name the pathogenic organism and describe the disease. **148**
- ☐ 3 Give examples of plant diseases caused by pathogens. For each example, name the pathogenic organism and describe the disease. **149**
- ☐ 4 Give examples of diseases caused by bacteria. For each example, name the bacteria and describe the disease it causes. Describe the shapes (morphology) of bacteria and provide examples of each. Describe common modes of bacterial transmission (foodborne, waterborne, and airborne), providing examples of each. **150**


NIH


CDC



- ☐ 5 Give examples of diseases caused by fungi. For each example, name the fungi involved and describe the disease it causes. Explain why fungal diseases are difficult to treat. **151**
- ☐ 6 Give examples of diseases caused by protists, with a particular focus on malaria (caused by protistan parasite *Plasmodium*). Understand and describe the role of the *Anopheles* mosquito as a vector for *Plasmodium* transmission. **152**
- ☐ 7 Describe the varied structure of viral pathogens. Provide examples of diseases caused by viruses. For each example, name the virus involved and describe the disease it causes. Explore Covid-19 as a case study of a viral disease and analyse secondary-sourced data about the impact of Covid-19 in Australia. **153**
- ☐ 8 Understand that prions are mutated, misfolded, infectious proteins. Describe examples of prion diseases and explain how they spread through populations. **154**

Understanding disease transmission

Key skills and knowledge

- ☐ 9 Describe the routes pathogens can use to enter the body and the various ways they can be transmitted between hosts (direct contact, indirect contact, vector transmission). **155**
- ☐ 10 Define epidemic and pandemic. Understand the factors contributing to an epidemic and describe the factors contributing to disease spread (including the role of rapid transportation methods). Analyse the Ebola Virus Disease (EVD) case study to understand disease transmission during an epidemic. **156 157**
- ☐ 11 PRAC Design and carry out an investigation to test for microbial contamination in a named food or beverage. **158**
- ☐ 12 Investigate the work of Louis Pasteur (microbial contamination) and Robert Koch (Koch's Postulates) in explaining the cause and transmission of infectious disease. **159**
- ☐ 13 Assess the causes and effects of agricultural diseases in Australia (provide plant and animal examples). **160**
- ☐ 14 Compare and describe the adaptations different pathogens have for 1: gaining entry into a host and 2: spreading between hosts. **161**

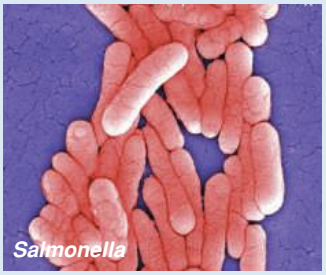
147 Pathogens and Infectious Disease

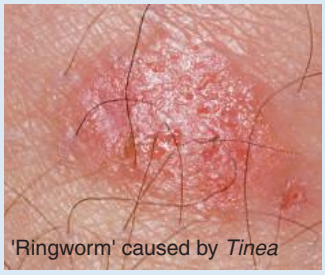
Key Idea: Pathogens are infectious agents that spread between organisms and cause infectious disease.

Pathogens are disease-causing agents and cause infectious disease. An **infectious disease** is a disease which can be spread between individuals. Pathogens can be classified in a number of different ways. Those which can be seen with the naked eye are called **macroorgansims**, while those which are too small to be seen with the naked eye are **microorganisms**. Pathogens can also be categorised depending on whether they are living organisms (**cellular pathogens**) or non-living organisms (**non-cellular pathogens**). Some common groups of pathogens are described below.

Cellular pathogens

Cellular pathogens are living organisms. They possess all the cellular machinery they need to carry out their life process and reproduce. Bacteria and eukaryotic pathogens (fungi, protists, and parasitic worms) are cellular pathogens. Cellular pathogens cause a wide range of diseases in plants and animals. The severity of the diseases they cause varies greatly: some have very mild effects (e.g. athlete's foot or ringworm), while some can cause death (e.g. TB or malaria).




'Ringworm' caused by *Tinea*


Malaria parasite (intracellular)

Bacterial pathogens

Pathogenic **bacteria** can be transmitted through food, water, air, or by direct contact. Bacteria have caused widespread, devastating diseases, but the discovery and use of antibiotics and aseptic (sterile) techniques has reduced deaths.

Fungal pathogens

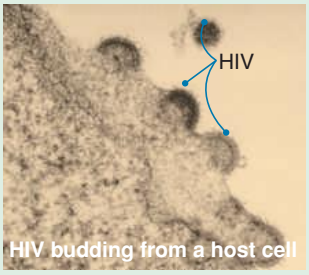
Pathogenic **fungi** are more common in plants than in animals. They spread by spores and the infections they cause are generally chronic (long-lasting, low grade) infections because fungi grow relatively slowly. However, some can be fatal.

Protistan pathogens

Protists are a large and diverse group of eukaryotes. A number of species are significant pathogens of animals or plants. Pathogenic protists have very complex life cycles, often involving a number of different hosts and several life stages.

Non-cellular pathogens

Non-cellular pathogens are non-living entities. Viruses and prions are both non-cellular pathogens. They do not have their own cellular machinery and must use a host's cellular machinery to reproduce.

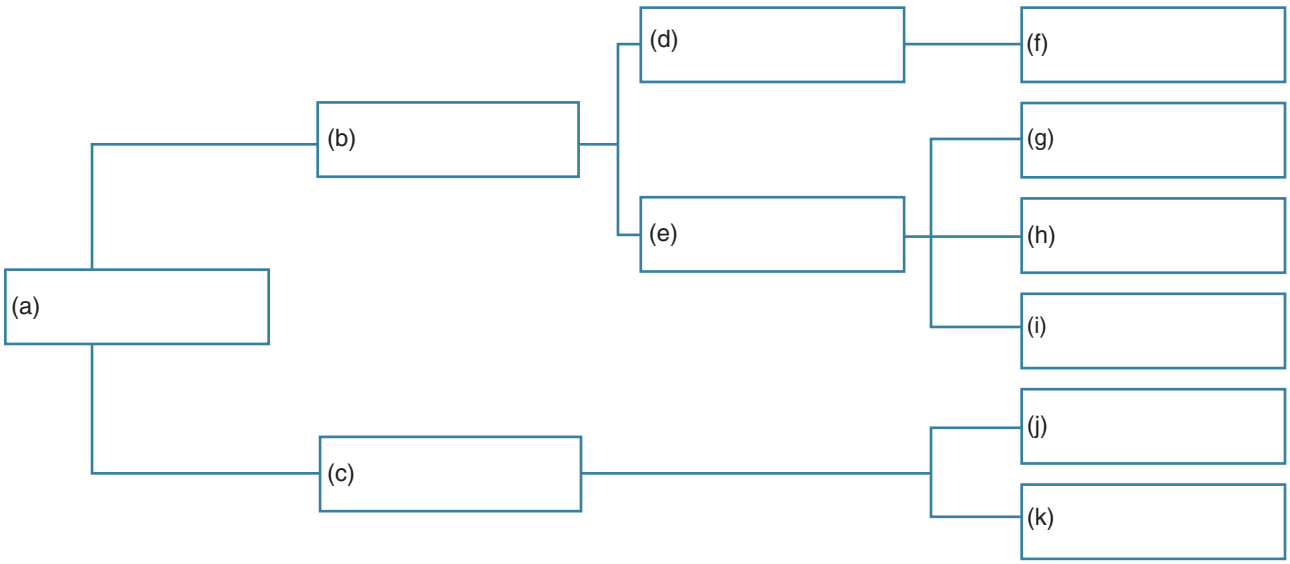


Viruses and prions

Viruses consist of a protein coat surrounding their genetic material. They cause a wide variety of diseases.

Prions are infectious, abnormal proteins which can damage other proteins. They cause a range of serious diseases.

1. Use the template below to categorise pathogens. Use the following word list to help you: *Cellular pathogen, virus, prokaryote, fungi, non-cellular pathogen, bacteria, pathogen, protist, parasitic worm, prion, eukaryote.*



2. Explain the difference between cellular pathogens and non-cellular pathogens: _____

_____

_____

_____

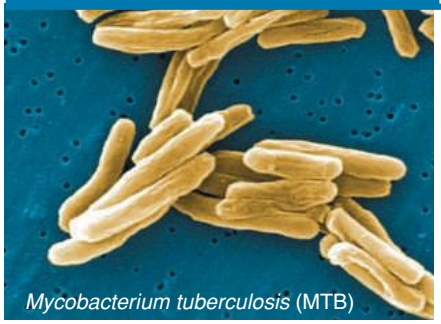
ISBN: 978-1-98-856655-9

148 Animal Pathogens

Key Idea: Animals can be infected by a wide range of cellular and non-cellular pathogens.

All animals can be infected by pathogens, although different species are more likely to be infected by some pathogens more than others. Animal pathogens can cause huge financial loss to farmers if livestock are infected, can affect the survival of a species, and can strain medical facilities during a large-scale outbreak. Around 1400 known pathogens affect humans, of which two thirds spread to humans from other animals (**zoonoses**). For instance, rabies can be transmitted from animals to humans. Pathogens also spread between humans, e.g. influenza, Covid-19, and Tuberculosis (TB).

Bacterial pathogens



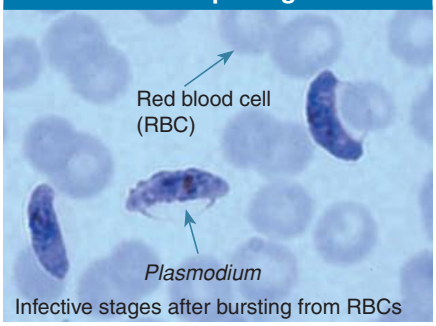
CDC: Janice Haney Carr

Mycobacterium tuberculosis (MTB)

Tuberculosis (TB)
TB is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (MTB). The pathogen is spread through the air when infectious people cough, sneeze, talk, or spit, and another person inhales the particles. MTB can enter a dormant phase in infected people, evading immune detection before becoming active again.

Kennel cough
Kennel cough is a respiratory disease in dogs (above). Several bacteria and viruses cause kennel cough but the bacterium *Bordetella bronchiseptica* is the main cause. It spreads when an infected animal coughs, and droplets containing the pathogen are transmitted through the air and inhaled by other dogs. Annual vaccinations are recommended to control the disease.

Protistan pathogens

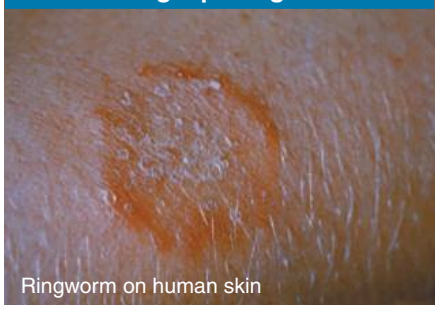


CDC

Infective stages after bursting from RBCs

Malaria
Malaria is caused by protistan parasites of the genus *Plasmodium*. The plasmodia have a life cycle involving two hosts, Anopheles mosquitoes, which act as a **vector** for transmission of the parasite, and humans. Humans become infected when bitten by infected mosquitoes. The plasmodia infect and multiply inside human red blood cells.

Fungal pathogens

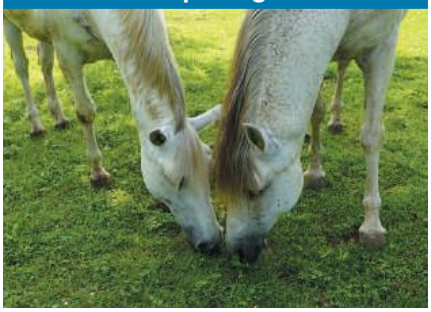


CDC

Ringworm on human skin

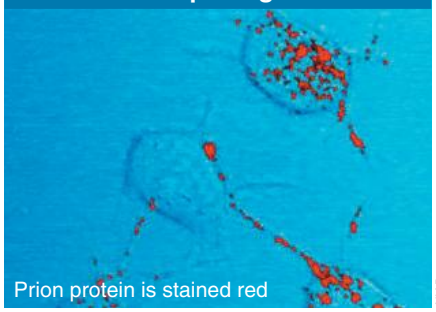
Ringworm and athlete's foot
A common fungal pathogen of the skin in animals is the genera *Trichophyton* which causes ringworm and athlete's foot. The ringworm fungus is spread by skin to skin contact. Athlete's foot is spread by spores. Fungal infections are slow-growing chronic infections making them relatively mild in most cases, but often difficult to treat.

Viral pathogens



Equine influenza
Equine influenza is caused by two subtypes of the Influenza A virus. It is a respiratory disease, and spreads easily between horses through coughing, sneezing, and touching (above). In 2007 an outbreak occurred at a quarantine facility in Sydney. Over 40,000 horses were infected, and it cost $571 million to eradicate the disease.

Prion pathogens



Prion protein is stained red

NIAD

Prion diseases
A prion is an abnormal protein which folds up the wrong way. Prions can trigger normal proteins in the brain to misfold, and they cause several fatal neural diseases in humans e.g. CJD (Creutzfeldt-Jakob disease) in humans and other animals. Eating contaminated meat is one way prion diseases spread.

1. Use the information above to complete the table below. Some cells have been filled in for you:

Category	Named pathogen	Disease	How is it spread?
Bacterial	(a)		Airborne, MTB are inhaled when an infected person coughs, sneezes, or spits.
Protistan	(b)		
Fungal	(c)	Amphibian Chytrid Fungus Disease	
Viral	(d) Subtypes of Influenza A virus		
Prion	(e) No specific prion named		

ISBN: 978-1-98-856655-9

149 Plant Pathogens

Key Idea: Plants pathogens include fungi, bacteria, protozoa, and some multicellular organisms.

A wide variety of pathogens are responsible for causing diseases in many plant species, including valuable crop plants. Plants can be infected by a single pathogen, but sometimes several pathogens can attack a plant at once. When this occurs it is called a **disease complex**. The combined pathogen attack and infections causes more damage than a single pathogen. Arthropods may act as vectors for the transmission of pathogens between plants. A **vector** is a living agent that carries and transmits an infectious pathogen to another living organism.

Viruses

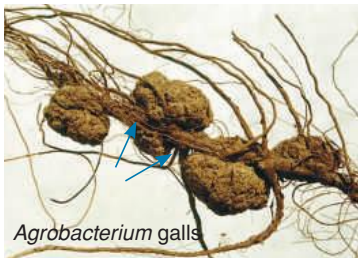
Viral pathogens cause a number of infectious plant diseases. Economically important plants that are affected include tobacco, potato, sugar beet, peach, elm, and orange. Symptoms include: stunted growth, colour change, malformations of leaves, or tissue death. Viral infections are spread by direct contact of diseased with healthy plants (e.g. during cropping) or via vectors such as sap-sucking leafhoppers. The TMV (below) is spread by contact.

Tobacco Mosaic Virus - TMV

WMU

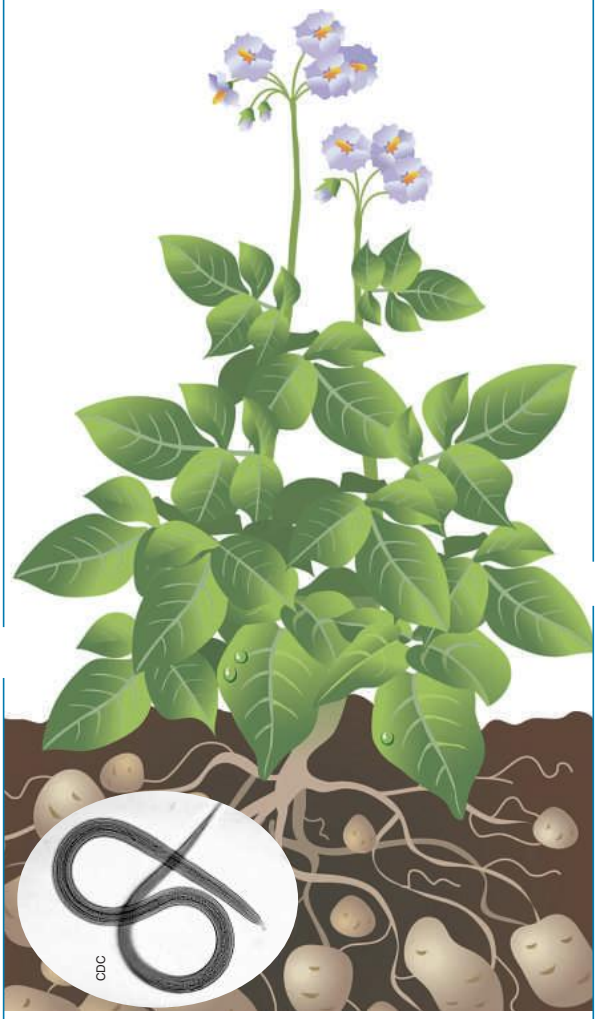
Bacteria

Over 150 bacterial species cause diseases in plants. Examples include: *Clavibacter michiganensis* which causes ring rot in potatoes and *Agrobacterium tumefaciens* which causes crown gall disease in a number of plants. Bacterial pathogens generally invade plant tissues though damaged tissue.



Agrobacterium galls

USDA



CDC

Nematodes (roundworms)

Plant parasitic nematodes (insert above) usually live in the soil and attack roots and tubers. A few species inhabit buds, stems, leaves, or flowers. More than 100 species cause stunting or death, and their economic impact is huge. Examples include the root lesion nematode and the citrus nematode.

Fungi

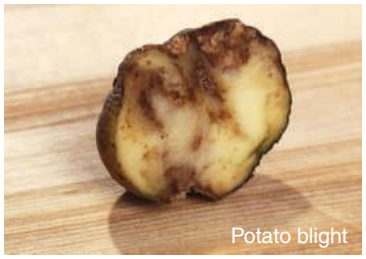
All economically important plants are affected by fungal diseases, with many affected by a dozen or more, e.g. black sigatoka or black streak disease (caused by *Mycosphaerella fijiensis*) produces black streaks and death of leaf tissue on banana plant leaves. It can eventually reduce crop yield by 50%. Fungal diseases are spread by spores, which germinate in warm, humid conditions.

Black sigatoka on banana

Fred Brooks, University of Hawaii cc 3.0

Protists

Potato blight is caused by the water mould parasite *Phytophthora infestans*. The motile spores of *Phytophthora* spread by wind or water in wet, warm, or humid conditions. Short-lived asexual spores can spread rapidly from host plants, whereas the sexual spores are long-lived and can remain viable for years. Potato blight causes damage to both the leaves and tubers of potatoes and can also infect tomato plants.



Potato blight

1. Why is a disease complex often more serious than infection with a single pathogen? _____

_____

_____

2. Describe briefly how each of the following plant diseases or pathogens is transmitted between plants:

(a) Tobacco Mosaic Virus: _____

(b) *Phytopthera* late blight: _____

(c) Crown gall disease: _____

(d) Black sigatoka: _____

ISBN: 978-1-98-856655-9

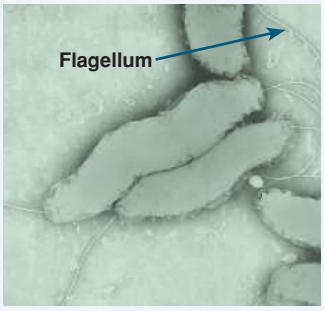
150 Bacteria

Key Idea: Pathogenic bacteria are responsible for some of the world's most serious diseases of plants and animals.

Although bacteria are very diverse in their metabolism, they generally fall into one of four basic shapes (below). Around 1400 bacterial species cause a wide range of diseases in humans. There are a number of ways people can become infected. For example, bacterial infections can be 'caught' from humans and other organisms, from touching contaminated surfaces, from contact with sewage or contaminated water, or from eating contaminated food. Bacterial diseases are commonly transmitted through food, water, air, or by direct contact. Controlling bacterial infection is achieved through identifying sources of infection, limiting its transmission, and treating the disease when it arises.

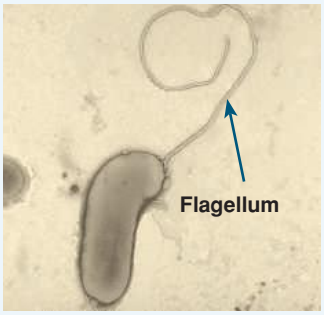
Bacterial shape

Bacteria generally fall into one of four general shapes.



EII

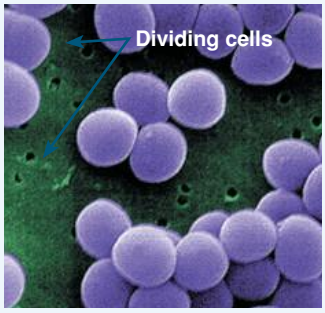
Spirilla: spiral shaped bacteria have a helical (corkscrew) shape which may be rigid or flexible. The *Campylobacter* above have a spiral shape.



Vibrios: curved or comma shaped cells, for example *Helicobacter pylori* above. The long flagellum allows it to move.

NAID

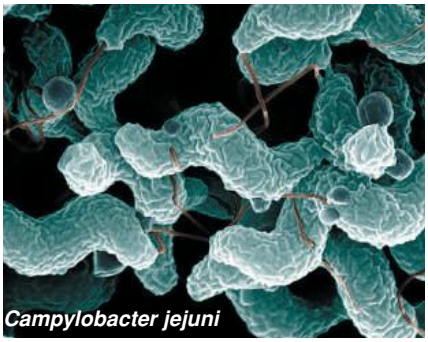
Bacilli: rod shaped, mostly occurring as single rods, but they may be found in chains or pairs. *Escherichia coli* (above)is a rod-shaped bacterium, common in the human gut.



CDC

Cocci: usually round like *Streptococci* or *Staphylococcus* above, but sometimes can be oval or elongated. When they divide, the cells stay attached to each other in pairs or clusters.

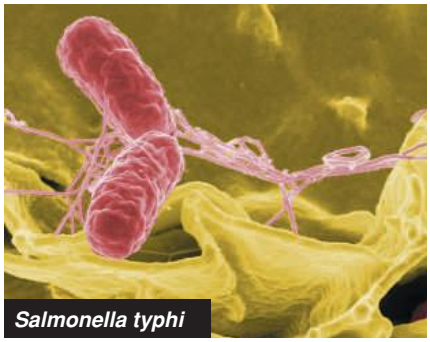
Methods of bacterial transmission



NIH

Foodborne bacterial diseases

Bacterial foodborne illnesses are caused by consuming food or beverages contaminated with bacteria or toxins produced by them. Examples include *Salmonella* and *Campylobacter* infection. Symptoms of bacterial food poisoning include fever, vomiting, and diarrhoea. Eating raw or under-cooked poultry is a common cause of food poisoning.



CDC

Waterborne bacterial diseases

Waterborne bacterial pathogens are responsible for a number of serious diarrhoeal illnesses, including typhoid and cholera. Transmission of these diseases is usually through faecal contamination of drinking water. The fever and diarrhoea associated with such diseases is responsible for hundreds of thousands of deaths annually in countries with poor sanitation.

CDC

Airborne bacterial diseases

Airborne pathogens are transmitted on droplets when people cough, sneeze, or exhale. Vaccination against some airborne bacteria, such as the respiratory disease whooping cough, has been highly successful. Immunisation reduces the number of infected or susceptible people, making it hard for the disease to be spread. Whooping cough is caused by the bacterial pertussis toxin.

1. Identify the four most common bacterial shapes, and provide examples:

   (a) _____

   (b) _____

   (c) _____

   (d) _____

2. There are currently around 30,000 known bacterial species. Of these 1,400 are pathogenic (cause disease) to humans. Calculate the percentage of pathogenic bacterial species:

   _____

ISBN: 978-1-98-856655-9

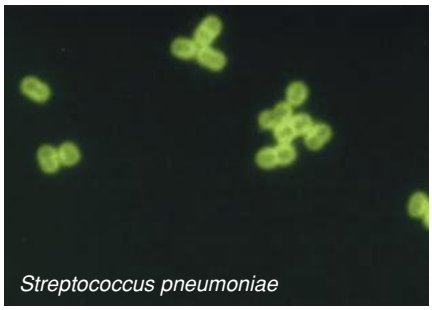
Examples of bacterial disease

Tetanus
Tetanus is a condition where the skeletal muscles undergo long, very strong and painful contractions. It is caused by the toxin tetanospasmin, produced by the rod shaped bacteria *Clostridium tetani,* a bacterium commonly found in the soil. Infection is usually through a puncture wound that becomes contaminated with soil or dirt.

James Heilman, MD CC 3.0

Puncture wounds on the feet are a common entry point for *C. tetani*

Bacterial meningitis
Bacterial meningitis is most commonly caused by the bacterial pathogens *Neisseria meningitidis* and *Streptococcus pneumoniae* (below). The bacteria infect the membranes around the brain (the meninges) causing headaches, fever, rashes, and sometimes death. Around 10%-20% of infected people will die.



Streptococcus pneumoniae

Plant crown gall
Crown gall is a tumour-like growth in plants caused by the soil bacterium *Agrobacterium tumefaciens*. The gall is produced when the bacterium transfers a circular piece of DNA (the tumour-inducing or *Ti* plasmid) to the plant cell. *A. tumefaciens* is now commonly used in biotechnology to insert new genes into plants for genetic modification.

USDA

3. Identify the three main modes of bacterial transmission and provide an example of each:

   (a) _____

   (b) _____

   (c) _____

4. (a) How does immunisation help control the spread of airborne bacterial diseases? _____

   _____

   _____

   _____

   _____

   (b) Give an example of an airborne bacterial disease well controlled by immunisation: _____

5. Why do you think it not a good idea to chop vegetables on the same chopping board used to prepare raw chicken?

   _____

   _____

   _____

   _____

6. Suggest why there are often outbreaks of bacterial diseases such as typhoid and cholera after large scale disasters, such as earthquakes or tsunamis:

   _____

   _____

   _____

   _____

7. Suggest why washing your hands before eating reduces the incidence of bacterial diseases:

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

151 Fungi

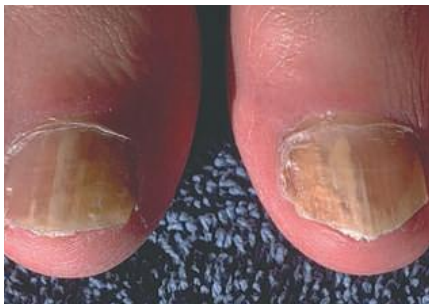
Key Idea: Pathogenic fungi are rare in animals, but they can cause infections that are long lasting and difficult to treat.

Very few **fungi** are pathogenic to animals, although thousands of fungal species are plant pathogens. They spread by spores and the infections they cause are generally **chronic** (long-lasting) infections because fungi grow relatively slowly. Fungal diseases are categorised into three broad groups (below), the most common being superficial infections of the skin. Of great concern is the spread of a fungal pathogen in amphibian populations. Amphibian chytrid fungus disease has been linked to the dramatic decline in amphibian populations globally. Amphibians rely on their skin for osmoregulation and oxygen uptake, so they are particularly vulnerable to pathogens that compromise this ability.

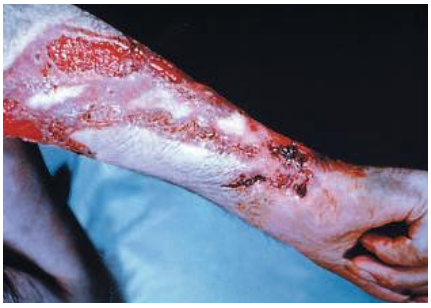
Types of fungal infection

CDC

Systemic infections: Usually occur deep inside the body, affecting internal organs, such as the lungs, bones, heart, and urinary tract. They often start in the lungs by inhalation of the spores and spread throughout the body. For example, candidiasis in the kidney above.



Cutaneous (superficial) infection: Infection that affects the skin, hair, nails, genital organs, and inside of mouth. Contracted through contact with spores, e.g. trichosporosis infection of the toenails above. They are slow growing and because of this they are difficult to treat.



CDC

Subcutaneous infection: Rare infection of the fatty connective tissue beneath the skin. Contracted through direct implantation of the spores into the skin via a scratch or puncture wound, e.g. sporotrichosis (above) caused by the fungus *Sporothrix schenckii*.

Chytridiomycosis

Chytridiomycosis is a waterborne disease of amphibians caused by the fungi *Batrachochytrium dendrobatidis*. It disperses via motile spores called zoospores, which enter the host via the skin, although much of how new hosts are infected is still unknown. It can be fatal to infected frogs within 10-18 days.

Batrachochytrium dendrobatidis is found in various parts of Australia, notably on the east coast, Adelaide, south-west Western Australia and the Kimberley region of WA.

Chytridiomycosis has been implicated in the dramatic population declines of frog species including the *Litoria nannotis* (waterfall frog) and *Litoria rheocola* (common mistfrog). It is also implicated in the extinction of at least four species of Australian frog.

Forrest Brem CC 2.5

Frog killed by chytridiomycosis. Note the reddening of the skin which is characteristic of the disease.

1. Describe two features of fungal diseases: _____

_____

_____

2. Why is it often difficult to treat a fungal infection? _____

_____

_____

3. (a) Suggest one way in which chytridiomycosis causes death in infected frogs: _____

_____

(b) Suggest why amphibians are so vulnerable to chytridiomycosis: _____

_____

_____

ISBN: 978-1-98-856655-9

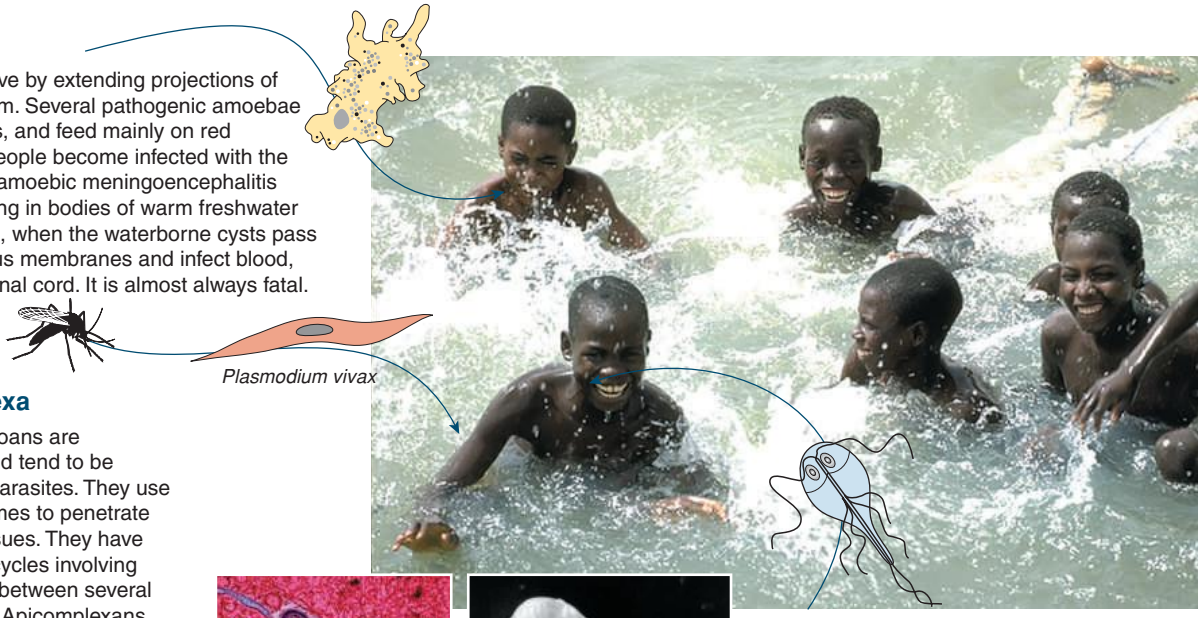
152 Protists

Key Idea: Protists are a large and extremely diverse group of eukaryotic organisms. A number of species are significant pathogens of animals or plants.

The protists are a group of unicellular or colony-forming eukaryotes. Most inhabit water or soil and only a few cause disease. However, a number of species are highly specialised pathogens. These include species of the parasitic genus *Plasmodium*, which cause malaria in humans, and species of the oomycete genus *Phytophthora*, which cause dieback and blight in a number of plant species. Pathogenic protists have very complex life cycles, often involving a number of different hosts and several different life stages. Both oomycetes and plasmodia, for example, have infective motile stages as well as resistant resting stages.

Amoebae

Amoebae move by extending projections of their cytoplasm. Several pathogenic amoebae infect humans, and feed mainly on red blood cells. People become infected with the pathogen for amoebic meningoencephalitis while swimming in bodies of warm freshwater or hot springs, when the waterborne cysts pass across mucous membranes and infect blood, brain, and spinal cord. It is almost always fatal.



Plasmodium vivax

Apicomplexa

These protozoans are not mobile and tend to be intracellular parasites. They use special enzymes to penetrate the host's tissues. They have complex life cycles involving transmission between several host species. Apicomplexans include *Plasmodium*, which is spread by mosquito vectors and causes malaria.

Plasmodium sporozoite stage moving through the cytoplasm of the intestinal epithelia.

Ute Frevert, Plos

Giardia trophozoite

CDC

Flagellates

Flagellates are usually spindle-shaped, with flagella projecting from the front end. The whiplike motion of the flagella pulls the cells through their environment. *Giardia* (left) is found in the small intestine of mammals. It is passed in the faeces and its life cycle alternates between an actively swimming trophozoite (left) and an infective, resistant cyst.

Phytophthora dieback

Phytophthora dieback is caused by the soil-borne water mould *Phytophthora cinnamomi*. Although it was originally classified as a fungus, *Phytophthora* is now included in the Protista. Flagellated zoospores enter the plant near the growing tip of the roots where they germinate, produce fungal-like hyphae, and absorb carbohydrates in the root. This eventually destroys the internal structure of the roots and causes the death of the plant. *Phytophthora cinnamomi* is one of the world's most invasive plant pathogens. In Australia, it is responsible for the dieback of *Eucalyptus* trees, especially in the Jarrah Forest bioregion of Western Australia. *Phytophthora* dieback can be treated with various fungicides including the use of phosphite salts (e.g. calcium phosphite).

Edward L. Barnard, Florida Department of Agriculture and Consumer Services, Bugwood.org

Phytophthora infection in a pine tree. Note the stunted growth. High water tables and excess irrigation provide suitable conditions root infections.

John H. Ghent, USDA Forest Service, Bugwood.org

Phytophthora infection in a pine tree showing rotted area near roots. Once a host is infected, water flow through the xylem is reduced via wilt-inducing toxins.

1. Several parasitic protozoans causing diseases in humans use other animal species as hosts for part of their life cycle. Identify the primary host that is involved in part of the life cycle for malaria:

_____

2. Why does infection by *Phytophthora cinnamomi* cause stunted growth and death in plants? _____

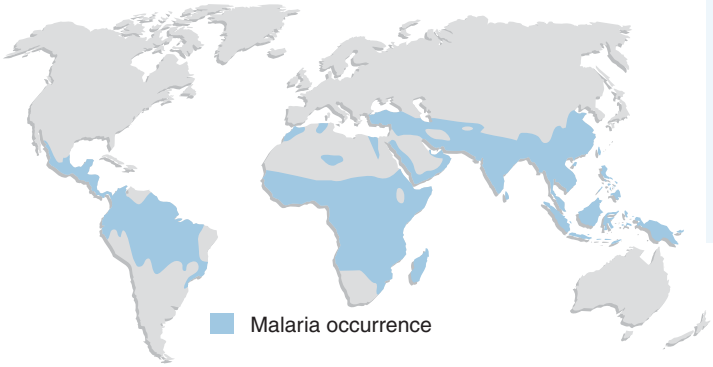
_____

_____

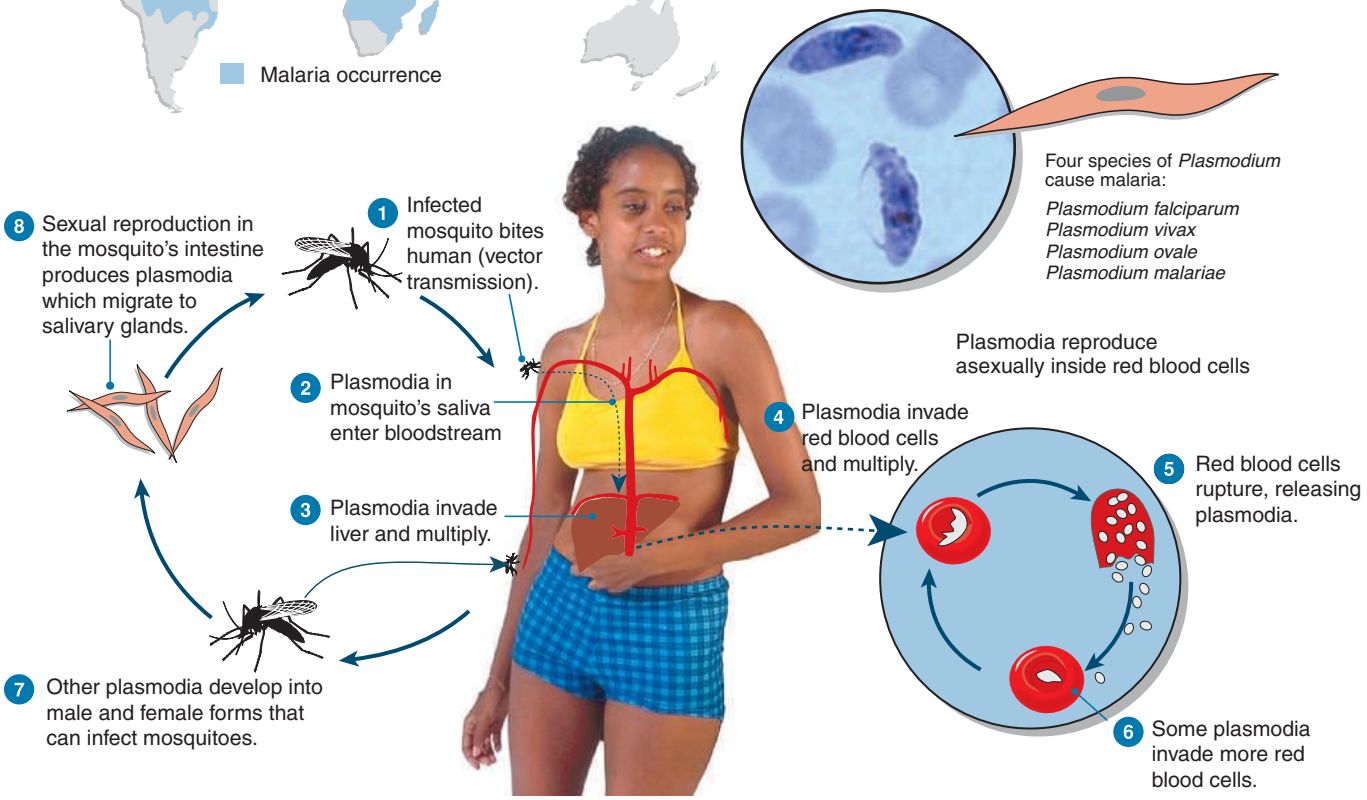
ISBN: 978-1-98-856655-9

Malaria is caused by a protistan parasite

- **Malaria** is a disease caused by protistan parasites of the genus *Plasmodium*. The plasmodia have a life cycle involving two hosts: *Anopheles* mosquitoes, which act as a **vector** for transmission of the parasite; and humans. Humans become infected when bitten by mosquitoes infected with the protozoans. In their human host, the plasmodia infect red blood cells (RBCs) and multiply inside the cells by asexual reproduction.
- Four *Plasmodium* species cause malaria, ranging in severity from relatively mild to fatal. *Falciparum* malaria is the most severe because it affects red blood cells of all ages. Destruction of the RBCs results in a condition called haemolytic anaemia (loss of RBCs through lysis). Infected blood cells also become sticky and block blood vessels to vital organs such as the kidneys and brain.
- Two strategies have helped control malaria. The first is the use of preventative antimalarial drugs. The second is vector control. This has involved spraying insecticide to kill mosquitoes and using insecticide-treated nets to prevent mosquitoes for biting people while they sleep.



Malaria is a major health problem in tropical regions where the climate is warm and wet enough to support breeding populations of the mosquito vector. Malaria affects more than 300 million people a year in equatorial regions (left). Cases in Australia only result when infected travellers return from these regions, although Northern Australia could harbour malarial mosquitoes if the global climate warms significantly.



3. How does a *Plasmodium* parasite enter the body? _____

_____

4. What aspects of the biology of this pathogen could make it difficult to control? _____

_____

_____

5. What biological factors are important in the global occurrence of malaria? _____

_____

_____

6. Why is global warming expected to increase the geographical range of malaria? _____

_____

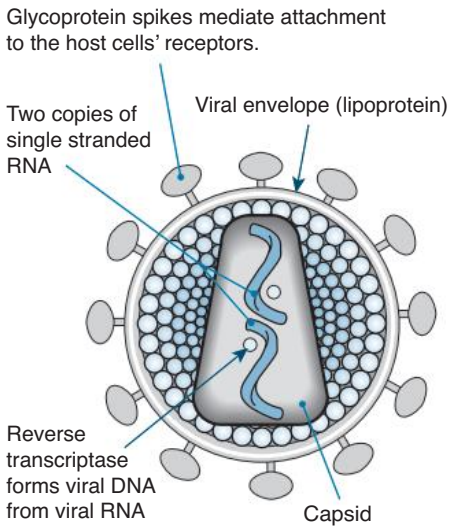
ISBN: 978-1-98-856655-9

153 Viruses

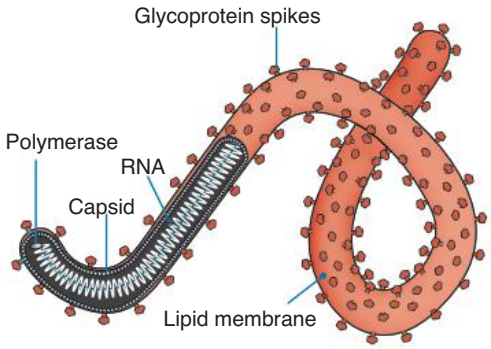
Key Idea: Viruses are infectious, highly specialised intracellular parasites. They are acellular and non-living.

Viruses are disease-causing agents (pathogens), which replicate (reproduce themselves) only inside the living cells of other organisms. Viruses are acellular, meaning they are not made up of cells, so they do not conform to the existing criteria upon which a five or six kingdom classification system is based. A typical virus contains genetic material (DNA or RNA) encased in a protein coat (capsid). Some viruses have an additional membrane, called an envelope, surrounding the capsid. Many viruses have glycoprotein receptor spikes on their envelopes that help them to attach to the surface of the host cell they are infecting. Viruses vary greatly in their appearance and the type of host they infect (below).

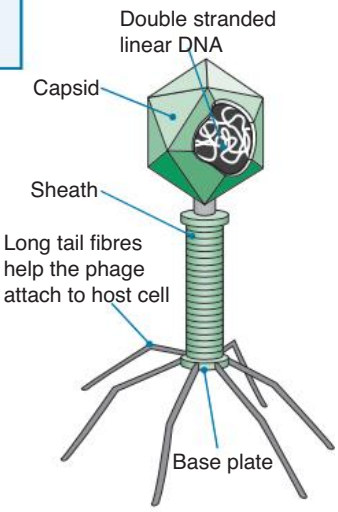
Viruses are not organisms! Viruses are metabolically inert until they are inside the host cell and hijacking its metabolic machinery to make new viral particles. However, they are often called microorganisms.



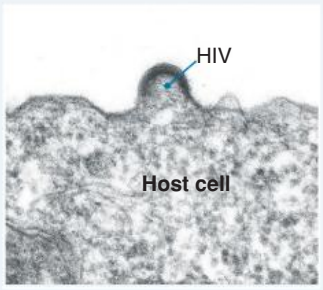
Structure of HIV, an enveloped retrovirus.



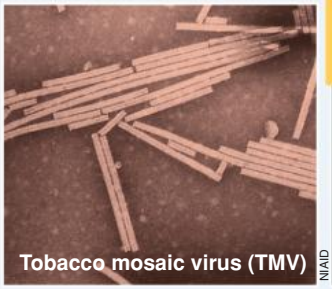
Structure of Ebola virus, an RNA filovirus that causes Ebola haemorrhagic fever.



Structure of Lambda phage, a bacteriophage that infects E.coli.

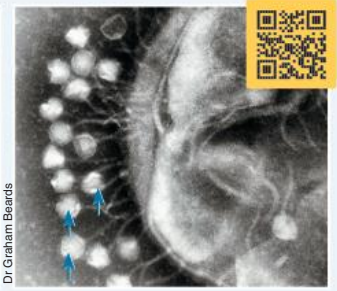


CDC



WMU

NIAID



Dr Graham Beards

After replication, new viral particles (**virons**) leave the host cell to infect more cells. In animals, enveloped viruses bud from the host cell, e.g. HIV (above left). Plant viruses cannot bud from the host cell due to the rigid cell wall. Instead, plant viruses, e.g. TMV (above right), move through the plasmodesmata connecting plant cells.

Viruses cause a wide variety of common human diseases, e.g. colds, influenza, Covid-19, AIDS and Ebola (above). Some are relatively harmless and others are life threatening.

Bacteriophages (arrowed) are viruses that infect bacteria. They use tail fibres to attach to the host cell and a contractile region below the capsid to inject their DNA into the cell.

1. Viruses are non-living. How do they replicate? _____

_____

_____

2. Describe the basic structure of a generalised virus, identifying the features they all have in common: _____

_____

_____

3. Describe the purpose of the following:

   (a) Glycoprotein spikes: _____

   (b) A bacteriophage's tail fibres: _____

   (c) Protein capsid: _____

ISBN: 978-1-98-856655-9

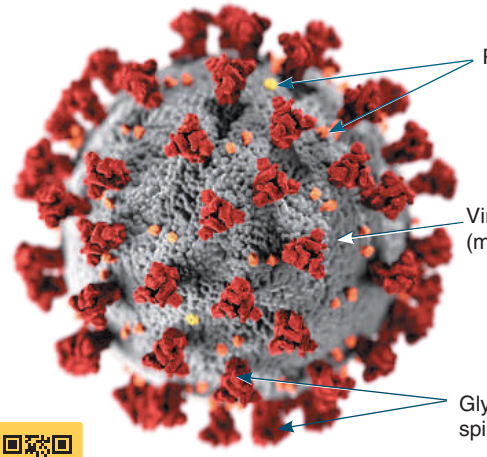
The emergence of Covid-19

- Reports of viral respiratory disease in Wuhan, China were reported on the 31st December 2019. Early in January 2020, a new coronavirus, SARS-CoV-2, was identified as the cause of the infections.

What is Covid-19?

- Covid-19 is the disease caused by infection with the SARS-CoV-2 virus (right).
- The virus affects the respiratory system.
- Most infected people recover without specialised care but some develop severe breathing problems and may require high level hospital care. The elderly, and people with underlying medical problems, are most at risk of becoming very sick.
- The virus is spread through the environment in small droplets from the nose and mouth (e.g. when a person speaks, sneezes, or coughs). People become infected when they breathe these droplets in, or when they touch a surface contaminated with the virus.
- Vaccines have now been developed and have helped reduce the severity of symptoms in many people.
- Several variations of the virus have emerged since 2019.

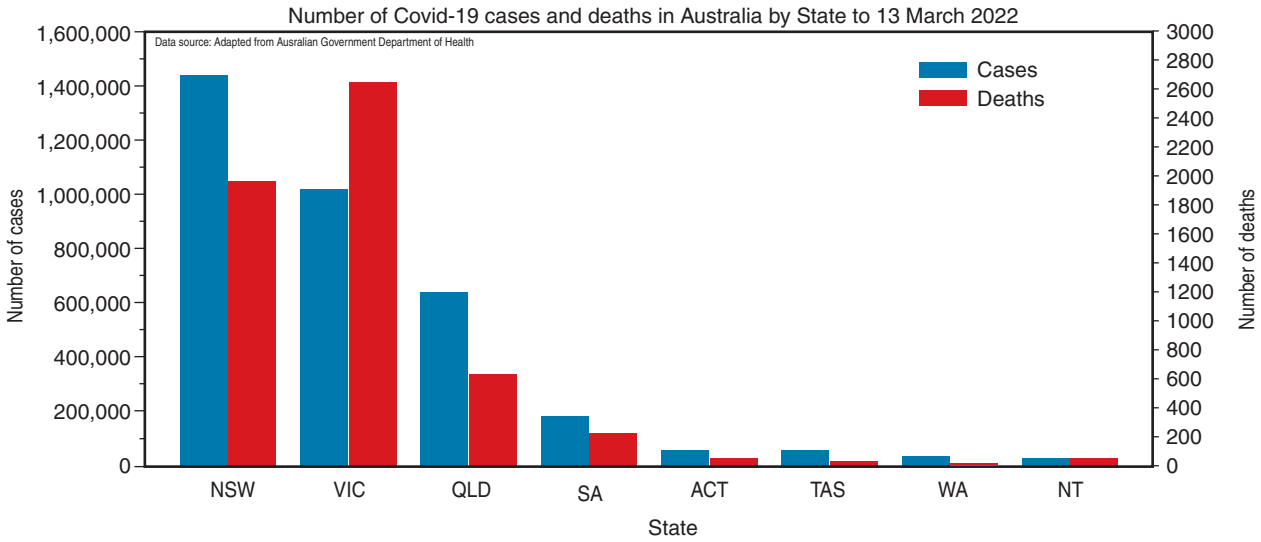
A representation of the SARS-CoV-2 virus



CDC/Alissa Eckert & Dan Higgins

Covid-19 in Australia

- Covid-19 first appeared in China towards the end of 2019. By early 2020 it had swept across the globe infecting millions of people and killing tens of thousands. As of March 2022, 457 million people have been infected with Covid-19 and six million people have died worldwide.
- Covid-19 was first reported in Australia in January 2020. Initially, the spread of Covid-19 in Australia was relatively limited, due to State and Federal Government moves to severely restrict travel both within the country and into the country.
- However, an easing of restrictions has resulted in subsequent waves of infection throughout most of Australia. To date (March 2022) there have been 3.6 million Covid-19 cases in Australia and around 5,500 deaths. A breakdown by state is provided below.
- Around 80% of the Australian population has been fully vaccinated (two doses of the vaccine) against Covid-19. The vaccination rate increases to 94% for Australians aged 16 and over.



4. (a) Name the virus that causes Covid-19: _____

   (b) How is the virus spread? _____

5. Use the graph above to compare how Covid-19 has affected NSW, compared to the other states: _____

ISBN: 978-1-98-856655-9

154 Prions

Key Idea: Prions are misfolded infectious proteins that can spread by causing misfolding in the original protein type.

Until recently, all pathogens were thought to contain some form of nucleic acid (e.g. DNA or RNA). We now know that some proteins, called **prions**, are capable of causing infection. Prions are spread by eating contaminated meat and, because they are difficult to remove by sterilisation methods, they can be spread on unclean surgical instruments. Prions are produced by mutations in the gene coding for a normal cell protein (PrP). They cause a group of degenerative nervous diseases in mammals called transmissible spongiform encephalopathies (TSE). They include **scrapie** in sheep, **BSE** in cattle, and **kuru** in humans. Different mutations of the PrP gene are responsible for each disease.

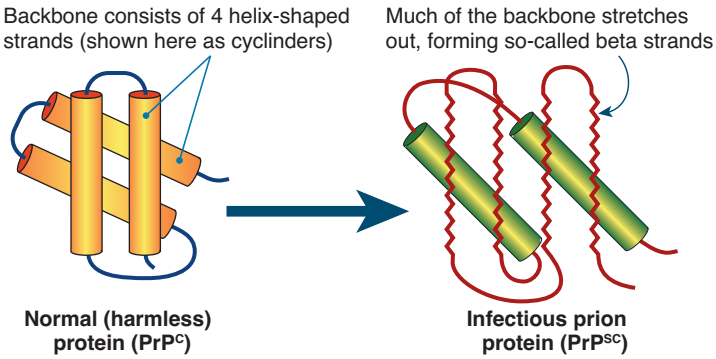
The disease **kuru** first brought prion diseases to prominence in the 1950s. It occurred in the geographically isolated tribes in the Fore highlands of Papua New Guinea. These people were eating the brain tissue of dead relatives for religious reasons.

Normal and infectious prions have the same primary structure, so go unchallenged by the immune system. If the infectious prion is from another species, as in vCJD, there is an initial immune response but this is shut down as the infectious protein converts more and more of the body's own PrP^{C} to PrP^{SC}.

Prion diseases of humans (and cause) *All these diseases are characterised by dementia and loss of coordination. There may be other symptoms as well.*
▸ Kuru (infection through cannibalism)
▸ Variant Creutzfeldt-Jacob Disease (vCJD) (infection)
▸ Classical Creutzfeldt-Jacob Disease (infection or mutation)
▸ Fatal Familial Insomnia (inherited mutation)

Infectious prion proteins

A shape change transforms the harmless protein into an infectious **prion**. The change may be caused by a point mutation in the encoding gene. The normal (common) form of the protein is denoted PrP^{C}, whereas the abnormal form is denoted PrP^{SC} (after scrapie, the prototype prion).

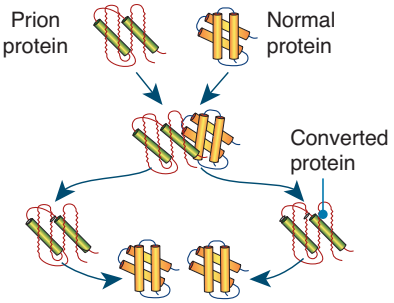


Propagation of the prion protein

Infectious protein (prion) has an unusual shape and binds to the normal form of the protein.

The normal protein is converted to the infectious prion.

The original and newly formed prions attack other nearby proteins. In time, prions accumulate to dangerous levels.



1. What main feature of prions distinguishes them from other infectious agents? _____

2. How does a prion's mode of transmission make it a successful agent of disease? _____

3. What is the source of infection for people with variant CJD? _____

4. How did the cultural practices of highland tribes in PNG enable the spread of kuru? _____

5. An epidemic of BSE in the UK in the 1990s had its origin in the practice of processing waste parts of cattle (particularly nervous tissue) and recycling them into cattle feed. Infected cattle subsequently entered the human food chain and were linked to cases of vCJD. Explain why it is poor practice to process an animal and feed it back to the same species:

ISBN: 978-1-98-856655-9

155 How Does Disease Spread?

Key Idea: Infectious disease can spread rapidly within and between geographical regions, given the right conditions.

The human body is constantly exposed to a wide range of potential pathogens. Transmission and spread of a pathogen depends on its rate of growth, the density of the host population, the mobility of the host population, and how the pathogen is transmitted between hosts. The transmission of infectious diseases can be virtually eliminated by carrying out good hygiene practices, providing adequate sanitation, and chlorinating drinking water.

Transmission and spread

Cough and fever are common symptoms of infection

Most pathogens, once inside the body, multiply rapidly, producing symptoms and making the host infectious within a few days. Others take longer to present symptoms. The infectious period can last from a few days to weeks, but in some cases the host may be infectious for long periods of time.

Human cities can contain millions of people, often living very closely together. In these overcrowded conditions infectious diseases can spread rapidly, especially if sanitation or personal hygiene is poor, or if seasonal weather produces conditions favourable to the spread of the pathogen. High speed transport (e.g. air travel) can help spread a pathogen around a region very quickly.

CDC

Child with the measles rash

The type of transmission (direct, indirect, or vector) affects how quickly a pathogen can spread and also how easy (or difficult) it is to control its spread. Spread is also dictated by how infectious the pathogen is. Highly infectious pathogens spread much more rapidly than others. For examples measles is much more infectious than the flu.

Portals of entry

Respiratory tract

The mouth and nose are major entry points for pathogens, particularly airborne viruses, which are inhaled from the expelled mucus of infected people.
Examples: covid-19, tuberculosis (TB), whooping cough, meningococcal meningitis, influenza, measles, rubella, chickenpox.

Influenzavirus

Salmonella typhi causes typhoid fever

Gastrointestinal tract

Food and water are often contaminated with microorganisms, but most of these are destroyed in the stomach.
Examples: cholera, typhoid fever, mumps, hepatitis A, poliomyelitis, salmonellosis.

Clostridium tetani causes tetanus

Breaking the skin surface

The skin provides an effective barrier to most pathogens, but cuts and abrasions allow pathogens to penetrate.
Examples: tetanus, gas gangrene, hepatitis B, rabies, malaria, and HIV.

Urinogenital openings

Urinogenital openings provide entry points for the pathogens responsible for sexually transmitted infections (STIs) and other opportunistic infections (i.e. thrush).
Examples: gonorrhoea, HIV.

HIV

1. Why can disease spread quickly in high density human cities? _____

2. Why would transmission by direct touch be slower than transmission by coughing or sneezing? _____

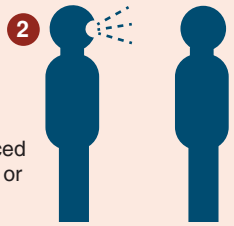
ISBN: 978-1-98-856655-9

Modes of transmission

Direct contact

Direct person to person contact can occur when an infectious person touches or exchanges fluids with another.

Person to person transmission (e.g. touching, kissing).



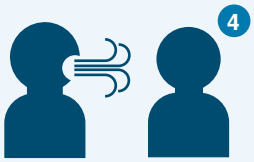
Contact with droplets produced when couching or sneezing.

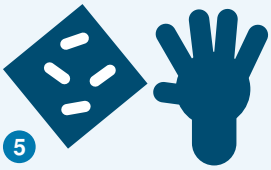
Indirect contact

Indirect transmission occurs when an infected person infects another without having direct contact with that person.

Faecal-oral transmission. e.g. someone not washing hands properly after using the toilet.

In some cases the pathogen can become airborne. Transmission can occur by breathing or coughing.





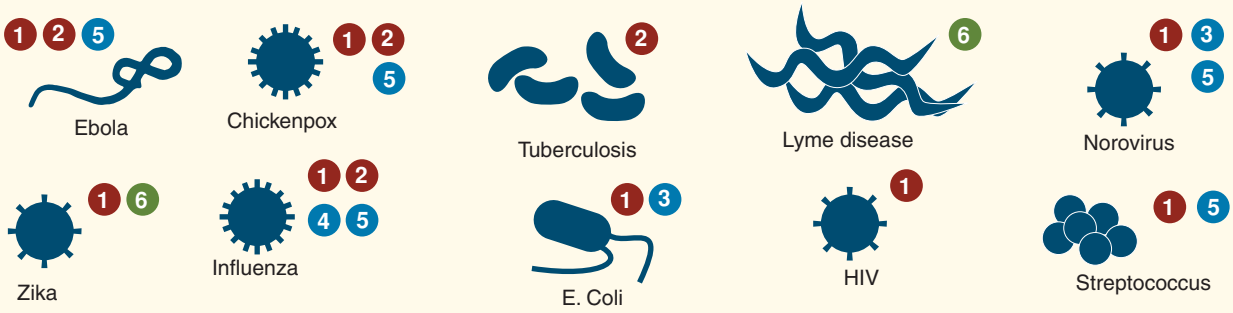
Transmission by contact with a contaminated object touched by an infected person.

Vector transmission

Vectors carry a pathogen from one person to another or from an animal to a human. Different vectors carry different pathogens.

Vector-borne pathogens are commonly carried by insects but sometimes other animals (e.g. ticks). They not only infect a person but can transfer the pathogen from person to person. The most well known example is malaria transmitted by the *Anopheles* mosquito.

Examples of transmission



Transmission of SARS-CoV-2

- The SARS-CoV-2 virus, which causes COVID-19, can be spread in the tiny liquid particles given off when an infected person coughs, sneezes, speaks, or breathes.
- Transmission can be by direct or indirect contact. Droplets may land on surfaces that are then touched by others. The virus needs to be breathed in, or make contact with the mouth or nose. A person may touch a contaminated surface with their hands then touch their nose or use their hands to eat and so become infected by the virus.
- The modes of transmission of SARS-CoV-2 make wearing masks and washing hands before eating important factors in slowing the spread of the virus.

3. Which disease is SARS-CoV-2 most similar to, in terms of its transmission?

_____

4. Why is coughing into your elbow more effective at stopping the spread of a virus than coughing into your hands?

_____

_____

5. How are vector-borne pathogens transmitted? _____

_____

_____

ISBN: 978-1-98-856655-9

156 Transmission of Disease During an Epidemic

Key Idea: The ability of a disease to spread and reach epidemic status depends on how easily the pathogen can spread, how resistant the population is to it, and how effective control measures are to contain it.

An **epidemic** is an increase in the number of cases of a disease above what is normally expected in a population. Often, the increase occurs suddenly. When the disease spreads over multiple countries or continents at once it is termed a **pandemic** (such as the Covid-19 pandemic). Disease outbreaks have occurred throughout history. The plague that spread through Europe in the 1400s (the Black Death) and again in the mid 1600s (the Great Plague) were some of the most devastating outbreaks ever. Today, medical and technological advances, disease modeling scenarios, and improved infrastructure and health services can be used to help predict and control disease transmission.

What causes an epidemic?

An epidemic of infectious disease occurs when a pathogen and its susceptible host(s) are present in sufficient numbers to allow the pathogen to move between, and infect, individuals reasonably quickly.

Epidemics can be caused by several factors:

- The pathogen has increased in numbers.
- The pathogen has become more infectious. This may be due to a genetic change (mutation).
- The pathogen has been introduced into an area or into a population where it has not previously been.
- The host has become more susceptible to the pathogen (e.g. through overcrowding, presence of other diseases, reduced immunity, low vaccination rates, poor sanitation).

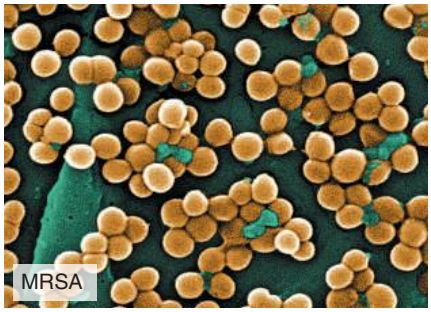
Predicting future disease is often a case of identifying diseases in animals that could cross over to humans. Many infectious diseases have an animal origin, including various strains of influenza (e.g. avian flu H5N1 and swine flu H1N1). Identifying these pathogens in animals, especially livestock and poultry living in close proximity to humans, can help prepare for possible outbreaks.

Population density is important to how quickly an infectious disease can spread. Cities generally have very high population densities, with some reaching densities of over 20,000 per km^2. Disease spreads most quickly in areas with poor living conditions, poor sanitation, and low levels of immunity. For example, the 1918 Spanish flu initially spread quickly due to the cramped conditions of military camps and hospital wards.

How quickly an infectious disease spreads also depends of the population's mobility. The 1918 Spanish flu spread around the world due to infected troops returning home and taking the disease with them. Part of predicting where and when diseases will occur is predicting the movements of groups of people. For example, people moving from rural areas to cities may transport potential pathogens from livestock.

All photos this row: CDC

The spread of disease also depends on the mode of transmission. Is it spread by airborne particles or by touch? The most feared scenario is an airborne pathogen that is highly contagious, has a long infectious period, and is ultimately deadly.



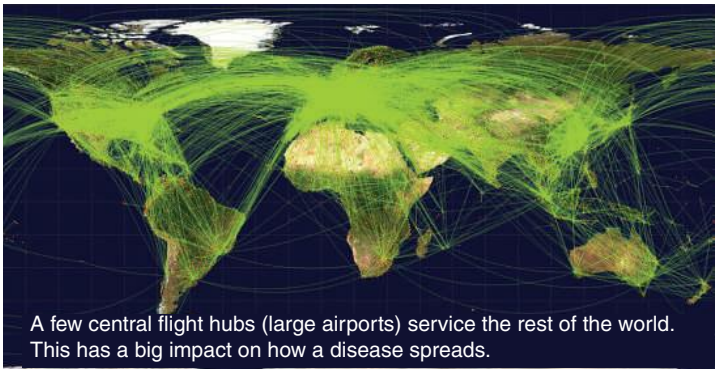
Resistance to antibiotics is becoming a greater problem, with many bacterial strains becoming extremely difficult to treat. Plans need to be in place for the spread of a highly resistant pathogenic bacteria through the general population.

Possible climate change is already being taken into account to predict the locations of possible outbreaks. For example, malaria may spread further north and south from the tropics as the climate becomes more favourable for its mosquito vectors.

ISBN: 978-1-98-856655-9

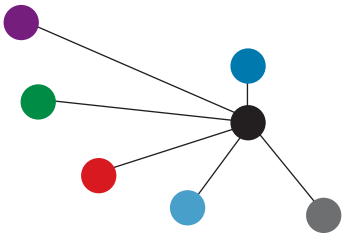
Predicting disease spread

- Understanding how a pathogen is transmitted and how quickly it can spread can help authorities plan and prepare for an increase in case numbers.
- In ancient times, the quickest way to reach a destination was to travel on land, usually by walking. Distance predicted how quickly a disease would spread. A disease often spread in a circular pattern from a central point or followed common trade routes.
- Today, it is not always distance that dictates the spread of a disease, but time. More efficient travel (cars, buses, trains, and planes) allow people to travel further, more quickly. If they are infected, the disease is also travelling at a more rapid pace.
- Global air travel routes often make it faster and easier to reach a destination by flying from a small hub to a larger one, then on to another small hub, rather than flying directly between two small hubs. For example, a person does not fly directly from Melbourne to Rio de Janeiro. Instead, a person flies to Sydney, then to San Francisco, then to Houston, then to Rio de Janeiro.
- The spread of a disease can be modelled based on the origin, where the major travel hubs are located, and the time taken to get between those hubs.

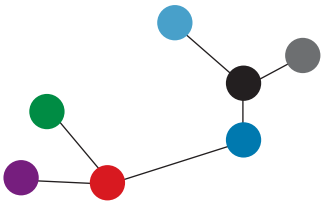


A few central flight hubs (large airports) service the rest of the world. This has a big impact on how a disease spreads.

Jpatokal CC 3.0



Based on distance, a travel map may look like this.



Based on flight times, a travel map may look like this.

1. Explain how each of the following are important in predicting where the next epidemic may originate:

   (a) Pathogen in livestock related to human diseases: _____

   _____

   _____

   (b) Population density: _____

   _____

   _____

   (c) Global travel networks: _____

   _____

   _____

   (d) Resistance to antibiotics: _____

   _____

   _____

   (e) Climate change: _____

   _____

   _____

2. In the boxes below, draw a diagram to show how a disease might spread, based solely on distance from the source, and then based on modern travel times from the source, as shown above:

Travel distance

Travel time

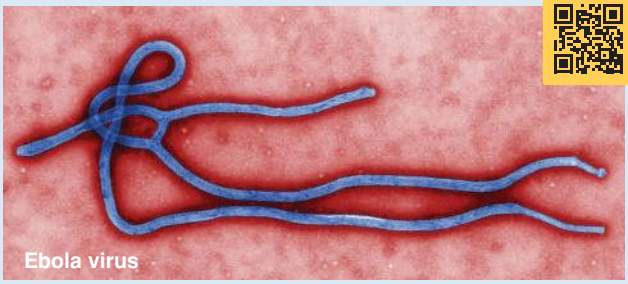
ISBN: 978-1-98-856655-9

157 Investigating Disease Transmission

Key Idea: Ebola Virus Disease can be used as a case study to investigate how disease spreads during an epidemic.

Studying how a disease spreads helps scientists and doctors understand how to control and reduce its spread. They can pinpoint the location of the outbreak and see how quickly the disease is spreading. Knowing where the disease comes from and how it spreads allows controls and procedures to be put in place to reduce transmission.

What is Ebola?



- **Ebola Virus Disease** (EVD) is a rare and deadly disease caused by a group of viruses within the genus *Ebolavirus*.
- EVD is also called hemorrhagic fever virus because it stops the blood from clotting. Without clotting, internal bleeding occurs as blood leaks out from the blood vessels. Death occurs in 50% of the cases, but can be higher depending on the virus.
- One particular virus, called **Ebola virus**, is responsible for many of the outbreaks of EVD in humans.
- Ebola virus is very infectious, it spreads easily when there is direct contact with the body fluids of an infected person or by touching items which have been contaminated with body fluids (e.g. bedding).
- Transmission between people can be controlled by practising good hygiene and not coming into contact with the body fluids or items of an infected person.

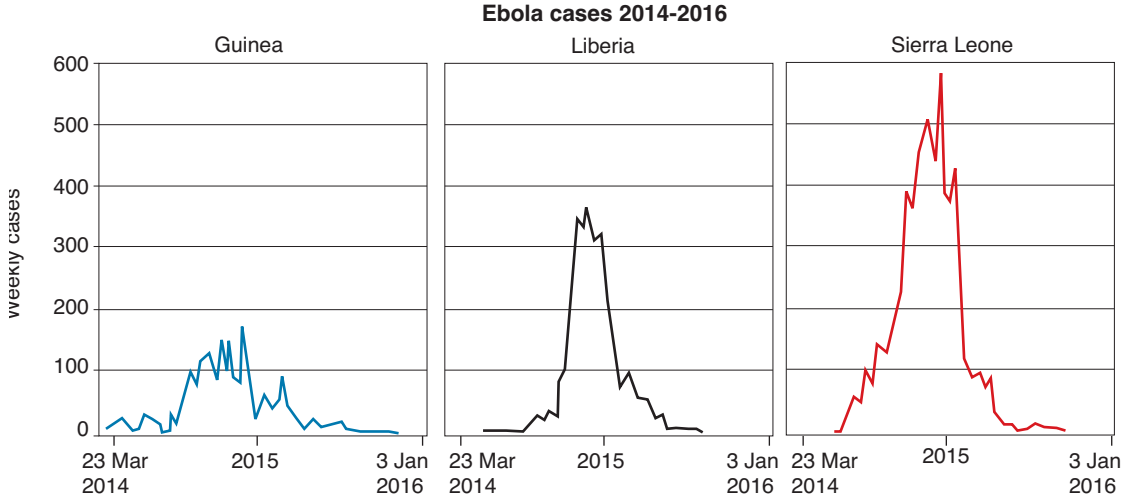
Case study: Tracking the spread of Ebola

Ebola virus disease (EVD) was discovered in 1976 when outbreaks occurred in Central Africa. The first outbreak occurred in the Democratic Republic of Congo in a village near the Ebola River, this is how the virus got its name.

Evidence suggested Ebola virus had existed in the environment before the 1976 outbreaks were observed. Population growth, people moving into forested areas and interacting with wildlife, and eating bushmeat may have contributed to the spread of the Ebola virus to humans.

Many outbreaks have occurred in Africa since 1976. The 2014-2016 West African epidemic started with a young boy becoming the first patient. It was suspected he had contracted the disease from bats. Weak reporting, limited controls on transmission, and an inadequate public health system response allowed the disease to spread to several countries. Some data regarding the 2014-2016 West African epidemic is provided below.

1. Study the graphs of the 2014-2016 West Africa Ebola outbreak below:



(a) In which country did Ebola first appear? _____

(b) Which country had the greatest number of cases of Ebola? _____

(c) What was the highest number of new cases reported per week? _____

(d) When and where did this occur? _____

2. Why did EVD spread so quickly and widely? _____

_____

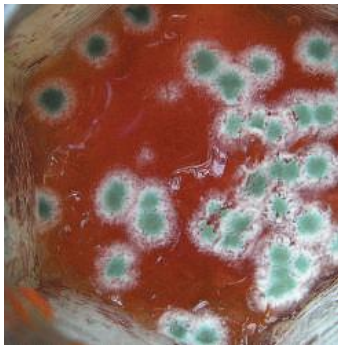
_____

ISBN: 978-1-98-856655-9

158 Testing Food for Microbes

Key Idea: Testing food for the presence of dangerous microbes ensures the food we consume is safe.

Food poisoning (foodborne poisoning) occurs when a person consumes food or water contaminated with a pathogen and becomes sick. Food poisoning is quite common, affecting 4.1 million Australians each year. Sometimes the illness is so severe a person needs to be hospitalised, and every year 80 people die from food poisoning. In Australia, food and beverages must meet strict testing guidelines to make sure the food is safe from microbial contamination. However, contamination can still occur. Sometimes this is the result of poor manufacturing, transport, or storage processes from the manufacturer or supplier, but most often it occurs due to poor food handling techniques at home. In this activity you will design and carry out a practical investigation to test for microbial contamination in food.



Sometimes it is easy to tell when food has been contaminated and shouldn't be eaten, like the spoiled jam, above left. However, most of the time microbial contamination is not so obvious, and people eat contaminated food and become sick. High risk foods include chicken, seafood, rice, eggs, deli meats, and dairy products.

Investigation 9.1 Investigating microbial contamination in food samples

See appendix for equipment list.

Treat all samples and equipment as though they contain pathogenic microorganisms. Wear protective eyewear and gloves. Wash surfaces and your hands thoroughly.

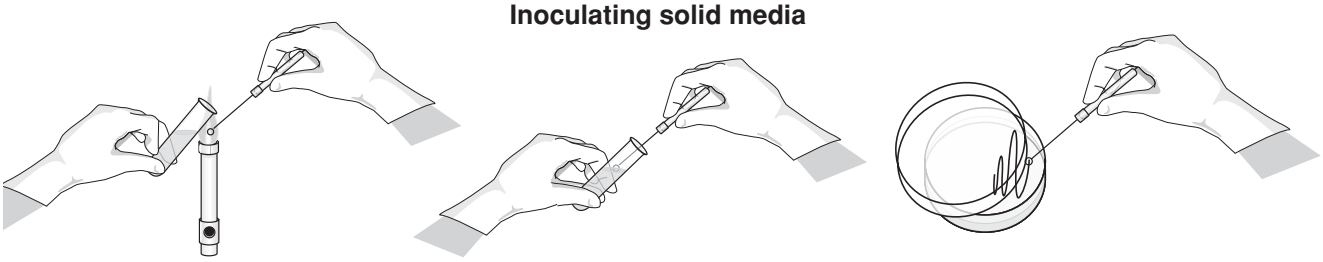
Design and carry out a practical investigation to determine the level of microbial contamination present in food samples. You may work in pairs or groups.

1. Decide what food you want to test (e.g. milk, meat, yoghurt etc).
2. Decide if you want to test the food simply for microbial contamination or do you want to do a comparative study. Comparative studies could include comparing microbial content of:
   - Refrigerated food or food left at room temperature
   - Cooked versus uncooked food
   - Food handled with unwashed hands vs washed hands
3. Design your own investigation using the following equipment list: food sample, agar plates, inoculation loops, Bunsen burner, sterilising alcohol, test tubes, glass rods, distilled water, tape, marker pens, incubator (if available). You may want to carry out some research to help you design your investigation. At the end of the investigation you should be able to count the number of fungal and bacterial colonies to determine the level of contamination.
   NOTE: It is important to exclude any environmental microbial contamination (e.g. from the air or your hands) when you carry out your investigation. Some information on aseptic technique is provided at the top of the next page.
4. Use the space below to plan your investigation. Use extra paper and staple it to this page if you need to.

5. Once you have completed your investigation, answer the questions on the following page.

ISBN: 978-1-98-856655-9

Inoculating solid media



1. Hold the inoculating loop in the flame until it glows red hot. Remove the lid from the culture broth and pass the neck of the bottle through the flame.
2. Dip the cool inoculating loop into the broth. Flame the neck of the bottle again and replace the lid.
3. Raise the lid of the plate just enough to allow the loop to streak the plate. Streak the surface of the media. Seal the plate with tape and incubate upside down.

1. Write a suitable aim for your experiment: _____

2. Record your method: _____

3. Describe your results: _____

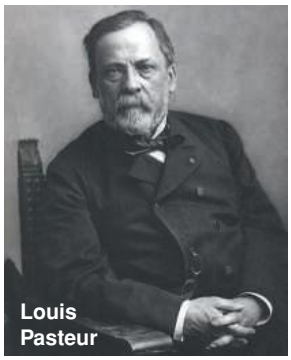
4. What can you conclude from your investigation? _____

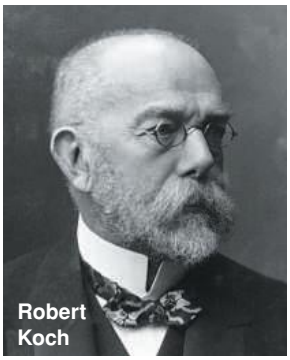
ISBN: 978-1-98-856655-9

159 Historical Understanding of Infectious Disease

Key Idea: The experiments of Louis Pasteur and Robert Koch contributed to our understanding of microbiology and infectious disease.

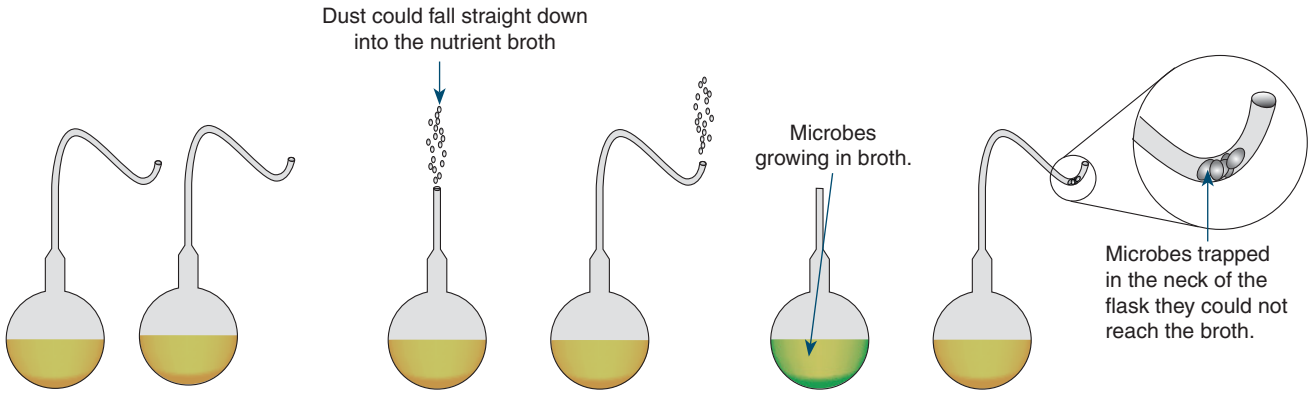
For a long time people thought that new life could spontaneously evolve from non-living matter (spontaneous generation) and that clouds of poisonous gas caused disease (Miasma Theory). The work of two scientists, Frenchman, **Louis Pasteur** and the German, **Robert Koch,** methodically disproved both of these theories. Their work contributed significantly to what we know about microbiology and the spread of infectious disease today.


Louis Pasteur


Robert Koch

Pasteur's swan neck flask experiment

Until the 1800s it was generally believed that new life could arise spontaneously from non-living matter. For example, when a piece of meat was left on a bench maggots appeared a day or two later. Under spontaneous generation theory the maggots had spontaneously generated from the components of the meat. Louis Pasteur disproved this theory when he carried out his very simple swan neck flask experiments (below).



1. Louis Pasteur filled two swan necked flasks with a nutrient broth and then boiled them to kill any microbes present.
2. He broke off the neck of one of the flasks allowing air and dust (on which microbes are carried) to fall straight down onto the broth. The neck of the other flask prevented dust falling onto the broth.
3. The broth in the broken flask eventually turned dark, indicating microbial growth. The broth in the unbroken flask remained unchanged. Pasteur concluded that spontaneous generation could not have occurred or both flasks would have turned dark.

1. A student set up an experiment to replicate Pasteur's experiment. Nutrient broth was added to two test tubes and the tubes were sealed. Both tubes were heated for several minutes in over a Bunsen burner. After cooling, tube 1 was uncovered, and tube 2 was left covered. The students observations are described in the table (right).

Test tube	Observation of the broth at day 1	Observation of the broth at day 10
1 uncovered	Clear	Cloudy
2 covered	Clear	Cloudy

(a) Did the student collect qualitative or quantitative data?

_____

(b) Explain your reason for your answer in 2(a): _____

_____

_____

2. Suggest a reason why the student's results were different from Pasteur's results: _____

_____

_____

_____

ISBN: 978-1-98-856655-9

The work of Robert Koch

Miasma theory proposed noxious clouds of gas (also called bad air or night air) contained diseases such as cholera or the black plague, and that people became sick by breathing in the gas. The theory had been popular and was commonly believed for many centuries until Robert Koch's work in the 1800s demonstrated that microorganisms caused disease. This finding became known as the 'germ theory'. Robert Koch's work with the anthrax bacterium allowed him to propose four basic criteria (known as **Koch's postulates**) for demonstrating that a disease is caused by a particular organism. These are described below.

Koch's postulates

1

Pathogenic microorganisms are isolated from a dead animal.

2

The microorganisms are injected into a healthy animal.

3

The disease is reproduced in the second animal. Microorganisms are isolated.

4

Isolated pathogenic microorganisms are identical to original pathogens.

Koch isolated bacteria from a diseased animal, then injected them into a healthy animal, causing it to exhibit identical symptoms to the first. This demonstrated that a specific infectious disease (e.g. anthrax) was caused by a specific microorganism (Bacillus anthracis). Koch used the procedure to identify the bacteria that caused anthrax and tuberculosis.

Koch's findings are summarised as Koch's postulates:

1. The same pathogen must be present in every case of the disease.
2. The pathogen must be isolated from the diseased host and grown in pure culture.
3. The pathogen from the pure culture must then cause the disease when it is inoculated into a healthy, susceptible animal.
4. The pathogen must be isolated from the inoculated animal and shown to be the original organism.

Postulate: to suggest a theory or idea as a basic principle from which a further idea is developed.

3. Carry out your own research and briefly outline how Louis Pasteur's discovery that heating kills microbes is used in food manufacturing processes today:

4. Carry out your own research and explain how the contributions of Louis Pasteur and Robert Koch have contributed to our understanding of microbiology and infectious disease:

ISBN: 978-1-98-856655-9

160 Causes and Effects of Disease in Agriculture

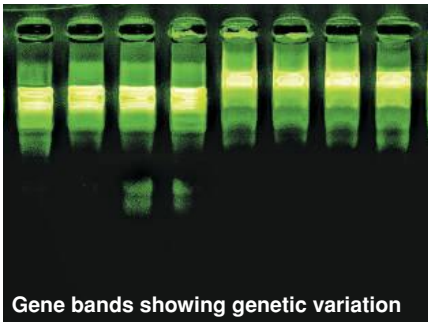
Key Idea: Agricultural production can be affected by a large number of plant and animal pathogens. Failure to control the pathogens can have serious outcomes.

Agriculture (farming) is the production of crops and livestock for food or some other resource (e.g. wool). It is important to the Australian economy as it creates jobs, generates income, and provides food for people and other animals. Infectious disease can spread rapidly through a farm. The disease may harm animal and plant health, reduce production (and sometimes cause food shortages) and reduce farm income. It is important that agricultural diseases are well understood to prevent them occurring or limit their spread. This requires knowledge about the pathogen, the effects of the disease, and how its spread can be controlled.

Diseases in crop plants

Healthy canola crop

Many different crops are grown in Australia. Some important crops include wheat, barley, maize, oats, banana, apples, canola (food and biofuel), and cotton (fibre and textile industry). The seriousness and infectiousness of disease that affects crops is influenced by pathogen type, health of the host plant, and environmental conditions (temperature, humidity, rainfall etc.), as these can physically stress the plant.



Gene bands showing genetic variation

Genetic makeup is an important factor in how a plant resists viral pathogens. The European Union group RESISTANCE found that plants with less genetic diversity were more likely to be infected and harmed by multiple viruses. Genetic modification (e.g. to increase yield) has seen the genetic diversity of many crop plants decrease. Some species are more likely to become infected, and, in extreme situations, this could lead to extinction.

Brown rot in peaches

Postharvest diseases occur after a crop has been harvested, often during transport or storage. Certain moisture and pH conditions cause pathogenic fungi and bacteria to grow and spoil the harvest. Steps can be taken to reduce post-harvest loss, such as controlling the crop's storage temperature and atmosphere (refrigeration or controlled atmosphere), using fungicides, washing with hot water, and irradiating to remove pathogens.

White mould

Rasbak CC 3.0

Disease: White mould or Sclerotinia stem rot

Pathogen: *Sclerotinia sclerotiorum*

Pathogen type: Fungi

Description: *Sclerotinia sclerotiorum* causes stem rot in many plants species, including beans and canola. Diseased plants have light-brown soft patches and a cotton-ball like growth (photo above). The infection spreads around the stem causing it to rot, and the plant dies.

White mould is more likely to develop if:

- *S. sclerotiorum* has been present in the area in the last three years (it can stay in the soil for many years and infect new crops).
- Infected broadleaf crops were grown in the same place the previous season (the fungi may still be present).
- Crops are planted in areas which stay wet and cool for long periods. These conditions are ideal for fungal growth and spore release.

Prevention: Sow clean seed, free of *S. sclerotiorum*. Spray with fungicide to kill the pathogen before it infects the plants. Plant in areas free of white mould disease.

Potato leaf roll

Green peach aphid feeding

Scott Bauer USDA

Disease: Potato leaf roll

Pathogen: Potato leaf roll virus (PLRV)

Pathogen type: Virus

Description: PLRV is common all over the world, including within Western Australian potato crops. Infection causes the potato plant leaves to curl up and roll inward, and the leaves may become brittle and change colour. Infection causes stunted (low) growth, and smaller or fewer potatoes are produced from a plant. Potato plants will die if the infection is severe enough.

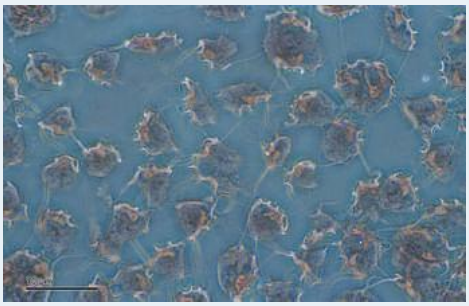
Potatoes are grown from the previous year's seed potatoes and, if the seed potato is infected when planted, it can act as a viral source to other plants. The virus is spread between plants by green peach aphids. The aphid feeds off an infected plant and spreads the virus to the next plant it feeds from. Tomatoes and capsicum may also be infected with PLRV.

Prevention: Test for PLRV in potato seed stock (tubers) before planting. Use insecticides to control green peach aphid numbers, if required. Remove self-sown (untested) potato plants as these may be infected with PLRV. Do not plant in areas with PLRV present.

ISBN: 978-1-98-856655-9

Diseases in livestock

Trichomoniasis



DOC. RNDr. Josef Reischig

Disease: Trichomoniasis

Pathogen: *Tritrichomonas foetus*

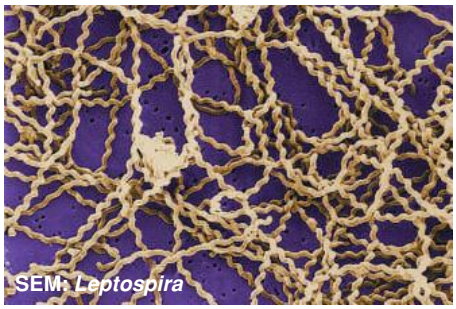
Pathogen type: Protist

Description: *Tritrichomonas foetus* is a mobile protistan parasite of cattle. It causes the sexually transmitted infection Trichomoniasis in cattle. The disease spreads during mating or through contaminated equipment used during artificial insemination. Most commonly, bulls infect cows, but an infected cow can infect a healthy bull too. The disease occurs throughout Australia, but is most common in Northern Australia.

Infected animals can be difficult to identify as there are few visible symptoms. Infected cows will have fertility issues, they may abort their calves, have difficulty getting pregnant, or be unable to become pregnant at all.

Prevention: The best preventative is testing new breeding animals for *T. foetus*. Any infected animals should be removed. Infected cows usually recover and are disease free after resting (no mating) for three months.

Leptospirosis



CDC: Rob Weyant

Disease: Leptospirosis

Pathogen: Genus *Leptospira*

Pathogen type: Bacteria

Description: Leptospirosis affects a wide range of livestock (cattle, sheep, goats). The disease is spread when an infected animal urinates on pasture, water, or feed supplies, and passes to other animals when they ingest the contaminated material. The kidneys are often affected by this disease. Symptoms include abortion or stillbirths in pregnant animals, reduced milk production, red-coloured urine, and death of younger animals. Animals can carry the bacteria for a long time without showing any symptoms, allowing it to spread widely. The disease is a major health and safety issue for farm workers who can also become infected if they are in contact with contaminated urine or materials.

Prevention: Vaccination is the best way to prevent a leptospirosis outbreak, but good farm practices, including regular health checks and making sure the animals are not overcrowded, are also important.

1. Identify how agricultural diseases can impact humans: _____

2. How does genetic diversity affect infection rates in plants? _____

3. Suggest why intensive livestock practices (i.e. high numbers of livestock being farmed) could increase the occurrence of disease on a farm:

4. Assess the causes and effects of disease on agricultural production. Provide an example for one plant pathogen and one animal pathogen. You should use the material on **BIOZONE's Resource Hub** or carry out your own research to supplement the information provided in this activity. Attach your answer to this page:

ISBN: 978-1-98-856655-9

161 Adaptations in Pathogens

Key Idea: Pathogens have a range of adaptations allowing them to successfully infect, reproduce, and transfer between hosts.

As we have seen earlier, there are many different types of pathogens infecting a wide range of hosts. Pathogens have a wide range of **adaptations** which allow them to gain entry, infect, and transfer between hosts. Some adaptations seem very simple, such as how the pathogen gains entry to the host (e.g. through a cut in the skin). Others are more sophisticated, such as having a metabolism which functions best at the host's body temperature, or adaptations for avoiding detection by the host's immune system.

General adaptations of bacterial pathogens

Around 1400 species of bacteria cause disease in humans. Each bacterium causes a different disease, and each bacterial species is highly adapted to increase its chances of successful infection and transmission. The diagram below is of a generalised bacterial cell, showing some of the general adaptations bacteria have evolved to be successful pathogens.
NOTE: Not all bacterial pathogens have all of these adaptations.

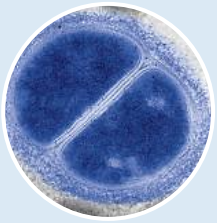
Varied transmission routes

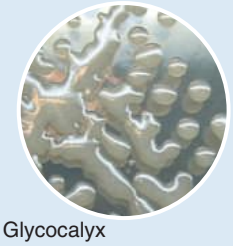
Bacterial pathogens have adapted many ways to transmit between hosts. Recall from earlier that this can include through air, water, food, and body secretions. Once in their hosts, some have special adaptations for infecting cells. For example, *Staphylococcus* bacteria (right) produce enzymes to break down blood clots. This allows the bacteria to move more easily around the body in the blood. Some *Staphylococcus* species secrete toxins that break down connective tissue between cells, allowing them to easily enter and infect a cell.

USDA

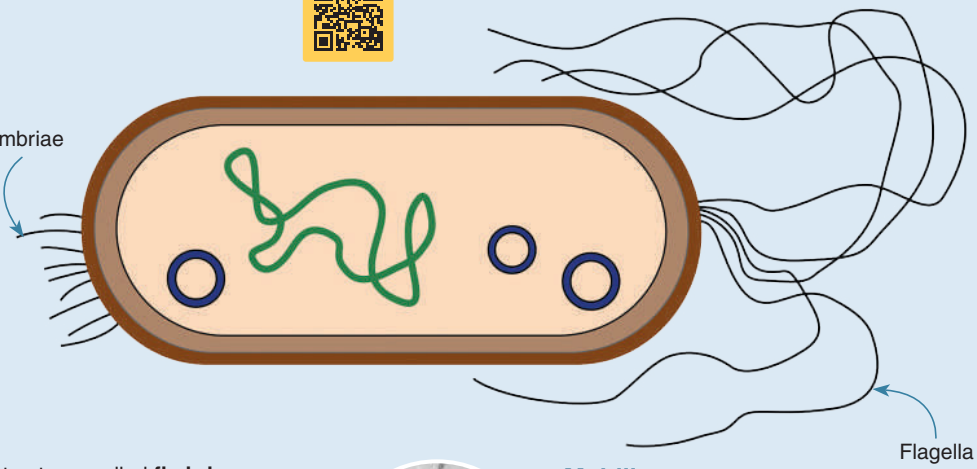
Rapid reproduction

Some bacteria multiply very rapidly. A single *Campylobacter* bacterium can multiply to 70 million cells in 12 hours! *Campylobacter* is a common food poisoning pathogen. A person becomes sick when they eat under-cooked, contaminated food (often chicken), or eat food prepared using contaminated equipment. Correct food handling techniques, proper cooking, and good hygiene are the best ways to avoid *Campylobacter* infection.



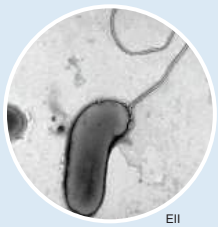


Glycocalyx



Attachment to surfaces

Some bacteria have hair-like structures called **fimbriae.** These allow them to attach to other cells (e.g. cells in the human intestine). Others have a **glycocalyx**, a sticky layer outside the cell wall, that can also be used to attach to cells. *Streptococcus pneumoniae*, which causes a number of lung diseases including pneumonia, uses a glycocalyx to stick to the lung cells. This makes it harder to remove the pathogen and can also protect the bacteria from the host's immune system.



EII

Mobility

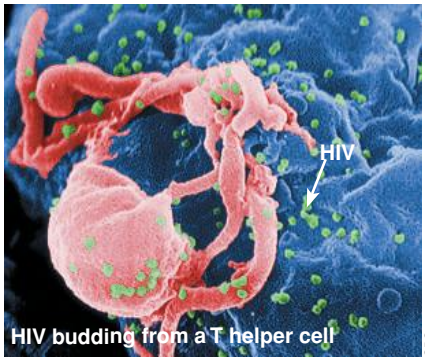
Some bacteria have flagellae that propel them through their environment. *Helicobacter pylori* (left) causes stomach ulcers in humans. Its flagellum allows it to move and helps it colonise and penetrate the lining of the stomach. *H. pylori* moves between people through direct contact with saliva, vomit, faecal matter, or contaminated food or water.

1. Briefly summarise some of the ways in which bacterial species have adapted to become successful pathogens:

ISBN: 978-1-98-856655-9

Human immunodeficiency virus

Human immunodeficiency virus (HIV) causes AIDS, a disease affecting the immune system. HIV infects and hides in T helper cells (a type of white blood cell) where it can avoid detection by the immune system and rapidly spread in the host. Usually, a viral infection triggers the production of a protein called interferon that 'interferes' with viral reproduction. HIV stops interferon production, and, with the anti-viral pathway out of action, the virus uses the cellular machinery of the T helper cells to rapidly reproduce. HIV is spread between people via the exchange of infected body fluids (e.g. blood, breast milk, semen, and vaginal secretions) and from contaminated needles.



HIV budding from a T helper cell

CDC

Australian paralysis tick

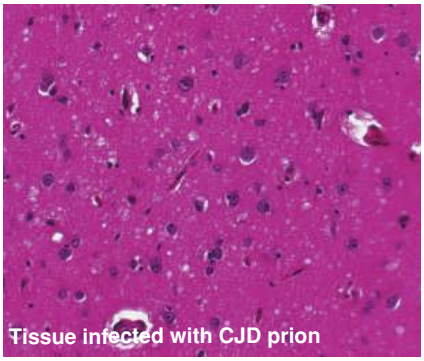
The Australian paralysis tick (*Ixodes holocyclus*) infects animals and people. The tick must feed off a host's blood in three separate sessions to complete its four-stage life cycle. It waits for its host to pass and attaches, feeding for 6-10 days each session. The tick secretes chemicals in its saliva to stop the inflammation reaction in the host (the tick remains attached, relatively undetected). They also inject neurotoxins (holocyclotoxins) designed to further disable the host's immune system so they can complete feeding. The neurotoxin can cause itching, anaphylactic shock, paralysis, and death in its host.


Australian paralysis tick (unfed and fed)

Bjørn Christian Tørrissen

Prions

Prions are faulty versions of the PrP^{C} protein. The misfolded form of the protein is called PrP^{SC} . PrP^{SC} has the ability to convert normal PrP^{C} into the abnormal form, i.e. it can infect other proteins. The prions gather together in large clumps, binding to the surface of a neuron and damaging it so that cell death occurs. Prions are easily transferred by eating contaminated meat or through infected medical equipment (recall that prions are resistant to sterilisation methods). Laboratory studies have shown that when prions are transferred to different host cells they undergo changes and adapt to the new cell host. This makes them a very dangerous infectious agent.


Tissue infected with CJD prion

DRdoubleB CC 3.0

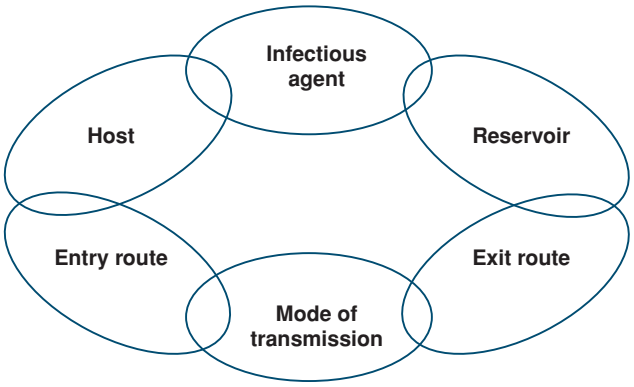
2. Compare the adaptations of two different pathogens that facilitate their entry and transmission between hosts:

ISBN: 978-1-98-856655-9

162 Chapter Review: Did You Get It?

1. Describe the difference between a pandemic and an epidemic: _____

2. The diagram (right) shows a simplified model of disease transmission.

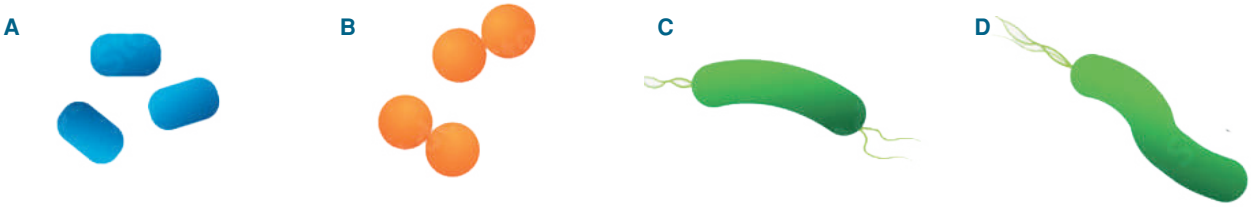


   (a) Pathogen A spreads very easily between people and its most likely entry route is through a person's nose and mouth. What is pathogen A's most likely mode of transmission?

   (b) Researchers suspect that a new pathogen (pathogen B) infecting humans may be transferred from bats to humans. What is the name given to diseases which spread from other animals to humans?

   (c) What would the researchers need to prove in order to confirm their suspicion about the transmission of pathogen B?_

3. The diagrams below represent the four common bacterial shapes. Name each shape and provide an example of a bacterial pathogen and disease for each group:



4. The table below lists some infectious diseases. Complete the table by naming the type of pathogen that causes the disease (bacteria, virus, protist), and the symptoms of the disease. You may need to do some extra research.

Disease	Type of pathogen	Symptoms of disease
Cholera	(a)	
Malaria	(b)	
TB	(c)	
HIV/AIDS	(d)	
Smallpox	(e)	
Measles	(f)	

ISBN: 978-1-98-856655-9

CHAPTER
10

Responses to Pathogens

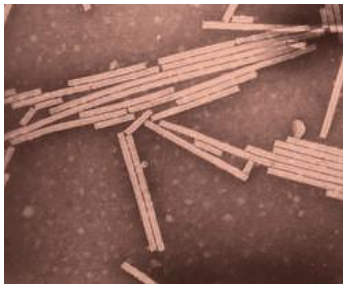
Key terms
active defence
allergen
antigen
innate immune system
interferon
interleukin-1
major histocompatibility complex
non-self antigen
passive defence
pathogen
RNAi
self antigen

Activity number

Inquiry question: *How does a plant or animal respond to infection?*

Plant and animal responses to pathogens

Key skills and knowledge



USDA

Edward L. Barnard, Florida Department of Agriculture and Consumer Services bugwood.org

163 Plant Responses to Pathogens

Key Idea: A plant's response to a pathogen involves both physical defences and chemical defences.

Physical defences in plants block pathogens from entering the plant. They include the bark of the tree, and the waxy cuticle on the leaf. Chemical defences, including antimicrobial and antifungal chemicals, may act internally or externally.

Chemical defences in plants

Passive defences

- **Passive defences** are always present and are not the result of contact with a pathogen or grazer. Plants have both physical and chemical defences to deter pathogens. If the physical defence is breached, the chemical defences protect the plant against further damage.

The powdery mildew infecting this plant is a fungus

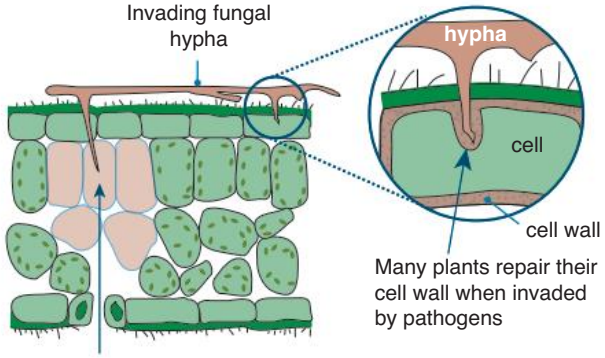
- Many plants produce a range of antimicrobial and antifungal chemicals and enzymes to kill or inhibit the growth of pathogens. Some of these compounds cover the surface of the plant, killing pathogens before they enter the plant. Other compounds act internally.
- Many native Australian plants have antimicrobial properties which are exploited by humans for medicinal purposes. The antimicrobial properties of the emu bush have long been used in traditional Australian Aboriginal medicine to treat infections of the skin, eyes, and throat. Four antimicrobial compounds have been isolated from emu bush.

Emu bush (*Eremophila alternifolia*)

Geoff Derrin CC 4.0

Active defences

- Once infected, a plant responds actively to prevent any further damage. **Active defences** are invoked only after a pathogen has been recognised, or after wounding or attack by a herbivore. This makes biological sense because active defences are costly to produce and maintain. Active defences work through a variety of mechanisms including slowing pathogen growth, puncturing the cell wall, disrupting metabolism, or killing cells by release of reactive oxygen species such as hydrogen peroxide (H_2O_2).



Many plants produce an enzyme-activated **hypersensitive response** when invaded by pathogens. This leads to the production of reactive nitric oxide and cell death. Cell death in the infected region limits the spread of the pathogen.

Sealing off infected areas gives rise to abnormal swellings called **galls** (oak gall, (left) and bulls-eye galls on a maple leaf, (right). These galls limit the spread of the parasite or the infection in the plant.

EII

Physical defences in plants

- Plants have physical defences that protect them from infection by pathogens.
- Many trees and shrubs are covered in bark. This tough external covering forms a physical barrier which pathogens find difficult to cross.
- The addition of pectin (a type of carbohydrate) and a layer of cellulose (the cell wall) adds strength and has the added benefit of providing extra layers to protect against infection.
- Many plants have leaves covered in a waxy cuticle. The cuticle is another physical barrier, reducing pathogen entry into the plant tissue via the leaf.

Juniper wattle

lookscloser CC 2.0

Hoya, an Australian native

Woody stems and bark

Trees and woody shrubs, like the juniper wattle, have thick coverings of waxy suberin which limit pathogen entry to the inner tissues. The spiny, reduced leaves protect it from being eaten, but also limit the pathways for pathogen entry.

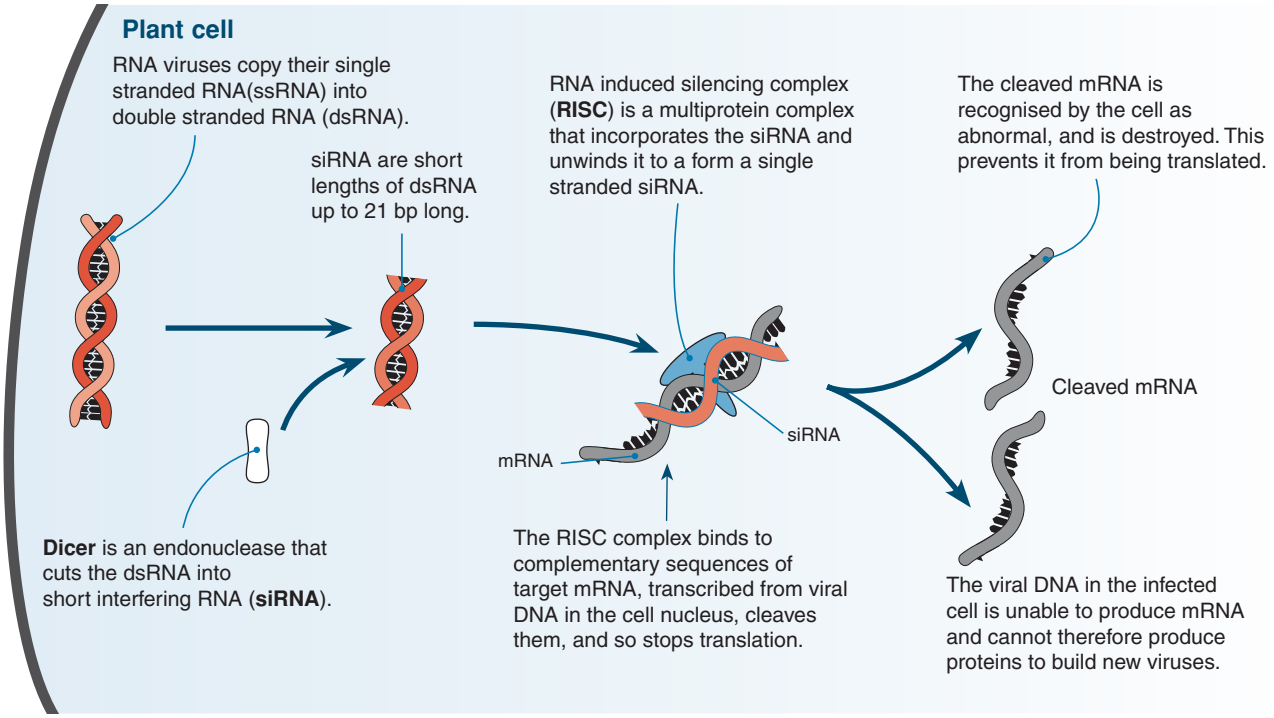
Impervious waxy cuticle

All plants have a waxy cuticle, even if it is thin. The cuticle is made up of fatty acids so all leaves are negatively charged and hydrophobic. These properties repel many spores and microbes and make the leaf environment less suitable for fungal invasion.

ISBN: 978-1-98-856655-9

Responding to viruses

- Although plants don't have an immune system like animals, they are able to produce a specific immune response. Plants have a special way of fighting viruses called RNAi or RNA interference (below). Infected cells are also able to send messages to neighbouring cells, which prepares them for the virus.



1. (a) Distinguish between passive and active defence mechanisms in plants: _____

   (b) Why are most plant defensive chemicals produced only after a pathogen is detected? _____

2. How are galls effective in reducing the spread of infection in some plants? _____

3. What features of physical barriers make them an important first line of defence against pathogens?

4. Some plants leaves secrete wax in response to the presence of pathogens. How is this different from the other physical barriers in plants?

5. How does RNAi provide plants with a type of immune response? _____

ISBN: 978-1-98-856655-9

164 Investigating Plant Responses: Pathogens

Key Idea: Plant pathogens produce a wide range of effects and responses in plants.

Plants are continually under attack from pathogens. In Australia, the accidental introduction of virulent pathogens potentially threatens both native plants and economically important plant crops.

Invasion of myrtle rust *Austropuccinia psidii*

- *Austropuccinia psidii*, or myrtle rust, is native to South America. It first appeared in Australia in 2010, in New South Wales. It has since spread to along the east coast of Australia to the far north of Queensland.
- Plants of the Myrtaceae family (myrtles), which includes eucalypts, the major plant form in Australia, seem to be particularly susceptible. Plants such as the native guava have suffered severe dieback events due to the spread of the fungus.
- A myrtle rust infection has a characteristic yellow centre with purple edges.

Myrtle rust on broad-leaved paperbark.

John Tann CC 2.0

Effect of myrtle rust on rainforest structure

- A large proportion of Australian native vegetation belongs to the myrtle family and is, therefore, particularly susceptible to the effects of myrtle rust. The data below comes from a study of the forest canopy near the border of Queensland and New South Wales. It shows the % of plants with branch death and the % of the remaining branches with dieback.
- Note that regeneration refers to saplings and seedlings, under-storey to trees between 1 and 4 m tall, and mid-storey to trees between 4 and 15 m tall.

Tree species	Canopy position	% of canopy	Branch death %	Branch dieback %
Lilly-pilly *(Syzygium smithii)*	Regeneration	66	0.08	3.23
	Understorey	15	6.67	13.33
	Mid-storey	11	8	21.00
Rose myrtle *(Archirhodomyrtus beckleri)*	Regeneration	13	8.85	93.85
	Understorey	31	43.75	97.66
	Mid-storey	41	22.56	91.43
Silky myrtle (*Decaspermum humile)*	Regeneration	6	0	33.33
	Understorey	13	48.46	100
	Mid-storey	15	86.79	100
Scaly myrtle (Gossia hillii)	Regeneration	3	0	100
	Understorey	18	33.89	96.87
	Mid-storey	25	42.61	100
Smooth scrub turpentine *(Rhodamnia maideniana)*	Regeneration	12	2.92	100
	Understorey	19	4.74	93.42
	Mid-storey	0	-	-

Impact of *Austropuccinia psidii* (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland, Geoff Pegg *et al*, 2017

1. Describe the effect of myrtle rust on the following parts of the forest:

   (a) Regenerating plants: _____

   _____

   (b) The understorey: _____

   _____

   (c) The mid-storey: _____

   _____

2. How might myrtle rust affect the overall structure of the forest? _____

   _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

Investigating plant responses to pathogens

- Understanding plant pathogens and their effects are important parts of biology, conservation, and the economy. Pathogens that affect native species have the potential to threaten their survival, while pathogens that affect economically important crops have the potential to cost the country millions of dollars in lost productivity and sales.
- Investigate the effects of a named pathogen on a named Australian plant. Useful links can be found in the **BIOZONE Resource Hub**.

3. Identify a **native Australian plant** and a viral or fungal pathogen that affects it: _____

4. Does the plant have any protection against the pathogen? Explain: _____

5. What progression of infection occurs in the plant (what effect does the pathogen have on the plant)? Find an image of the plant showing the main infection. Print it out and paste it in the space provided:

6. Is the pathogen endemic or introduced? Is it causing any threats to the native plant population? _____

7. Identify an important **Australian plant crop** and a pathogen that affects it: _____

8. How is the plant affected by the pathogen? _____

9. Find an image of the plant, showing the main infection. Describe the infection; print the image and paste it below:

10. What is the economic impact of crop loss to this virus (e.g. dollars per year lost, % crop lost)? _____

ISBN: 978-1-98-856655-9

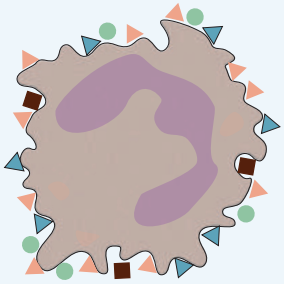
165 The Nature of Antigens

Key Idea: Antigens are substances capable of producing an immune response. It is important that the body can distinguish its own tissues from foreign material so that it does not attack itself.

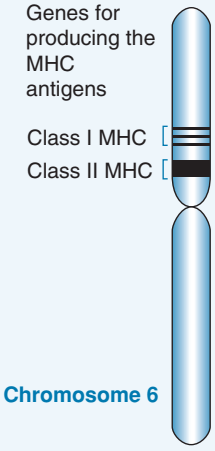
An **antigen** is any substance that produces an immune response. Most antigens are **non-self antigens**, i.e. they are foreign and originate from outside the organism (e.g. bacteria or viruses). Sometimes an organism will react to its own cells and tissues. Antigens that originate from within the body are called **self-antigens**. Normally, because of the development of self-tolerance, the body recognises and does not attack its own tissues. In some instances, the immune system may mistakenly destroy its own tissues. Such a response is called an autoimmune disorder. Allergens are a specific type of antigen that produce a vigorous hypersensitive allergic response.

Distinguishing self from non-self

- Every type of cell has unique protein markers (antigens) on its surface. The type of antigen varies greatly between cells and between species. The immune system uses these markers to identify its own cells (self) from foreign cells (non-self). If the immune system recognises the antigen markers, it will not attack the cell. If the antigen markers are unknown, the cell is attacked and destroyed.
- In humans, the system responsible for this property is the major histocompatibility complex (**MHC**). The MHC is a cluster of tightly linked genes on chromosome 6. These genes code for protein molecules (MHC antigens) that are attached to the surface of body cells. The main role of MHC antigens is to bind to antigenic fragments and display them on the cell surface so that they can be recognised by the cells of the immune system.
- Class I MHC antigens are found on the surfaces of almost all human cells. Class II MHC antigens occur only on macrophages and B-cells of the immune system.

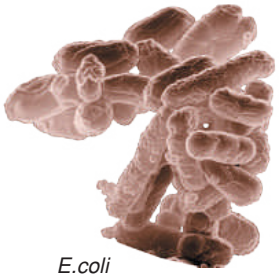


MHC surface proteins (antigens) provide a chemical signature that allows the immune system to recognise the body's own cells



Tolerance towards foreign bodies

- The human body has a very large population of resident microbes. Under normal conditions, *E.coli* in the gut form a protective layer preventing the colonisation of pathogenic bacteria. The microbial cells have foreign antigens but they are not attacked by the immune system because **tolerance** (the prevention of an immune response) has developed.



E.coli

- During pregnancy, specific features of the self recognition system are suppressed to allow the mother to tolerate a nine month relationship with a foreign body (the fetus).

Intolerance to tissue transplants

The MHC is responsible for the rejection of tissue grafts and organ transplants. Foreign MHC molecules on the transplanted tissue are viewed as antigenic, causing the immune system to respond and the tissue to be rejected. To minimise rejection, attempts are made to match the MHC of the organ donor to that of the recipient as closely as possible. Immunosuppressant drugs are also used to minimise the immune response.

Kidney transplant

1. (a) What is an antigen? _____

_____

(b) Distinguish between non-self antigens and self antigens: _____

_____

_____

_____

(c) Why is it important that the body detects foreign antigens? _____

_____

_____

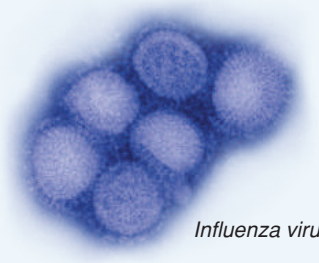
_____

ISBN: 978-1-98-856655-9

Types of antigens

Non-self antigens

Any foreign material provoking an immune response is termed a non-self antigen. Disease-causing organisms (pathogens) such as bacteria, viruses, and fungi are non-self antigens. The body recognises them as foreign and will attack and destroy them before they cause harm.



CDC

Influenza virus

Pathogens have ways of avoiding detection. Mutations result in new surface antigens, delaying the immune response and allowing the pathogen to reproduce in its host undetected for a time (e.g. the flu virus, above). Some pathogens, e.g. the malaria-causing *Plasmodium*, switches off its surface antigens in order to enter cells undetected.

Self antigens

The body is usually tolerant of its own antigens. However, sometimes the self-tolerance system fails and the body attacks its own cells and tissues as though they were foreign. This can result in an autoimmune disorder in which tissue is destroyed, grows abnormally, or changes in function.

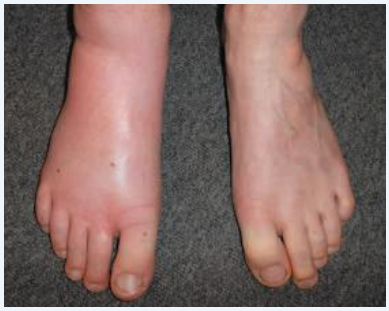
Autoimmune disorders, such as multiple sclerosis and rheumatoid arthritis, may be triggered by infection. The similarity of the pathogen and self antigens is thought to be behind this failure of self recognition.

Type 1 diabetes is the result of autoimmune destruction of the insulin-producing pancreatic cells. Patients must inject insulin to maintain normal blood glucose levels.

Allergens

Antigens that cause allergic reactions are called allergens. An allergic reaction is a very specific type of immune response in which the immune system overreacts to a normally harmless substance. An allergic response can produce minor symptoms (itching, sneezing, rashes, swelling) or life-threatening anaphylaxis (respiratory and cardiovascular distress).

Common allergens include dust, chemicals, mould, pet hair, food proteins, or pollen grains.



KP

The swelling on the foot in the left of the photograph is a result of an allergic reaction to a bee sting.

2. How can pathogens avoid detection by the immune system? _____

3. (a) What is the nature and purpose of the major histocompatibility complex (MHC)? _____

   (b) Why is a self-recognition system important? _____

4. (a) What is immune tolerance? _____

   (b) When might tolerance to foreign antigens be beneficial or necessary? _____

5. Using examples, describe what happens when the body develops an inappropriate response to:

   (a) Self-antigens: _____

   (b) Normally non-antigenic substances: _____

ISBN: 978-1-98-856655-9

166 Responses of Animals to Pathogens

Key Idea: Animals have a range of physical and chemical responses to the presence of pathogens.

Animals have developed a wide range of responses to defend against pathogens. Physical barriers stop the entry of pathogens. Chemical defences are activated if pathogens manage to enter the body. These include the production of many different types of enzymes that break down the cell walls of pathogens. Many of these chemical responses are innate or nonspecific and act against all types of pathogens.

Chemical defences in animals

Vertebrate innate defences

Antimicrobial substances: Chemicals (e.g. lactoferrin) are secreted from the skin and other body fluids, and also by some white blood cells. These kill pathogens or inhibit their growth.

Antimicrobial peptides: Defensins are abundant in phagocytes and small intestinal mucosa of mammals. They disrupt the plasma membrane of bacterial cells, causing death.

Lysozyme is an enzyme found in tears, saliva, milk, and mucus. It damages bacterial cell walls and the bacteria dies.

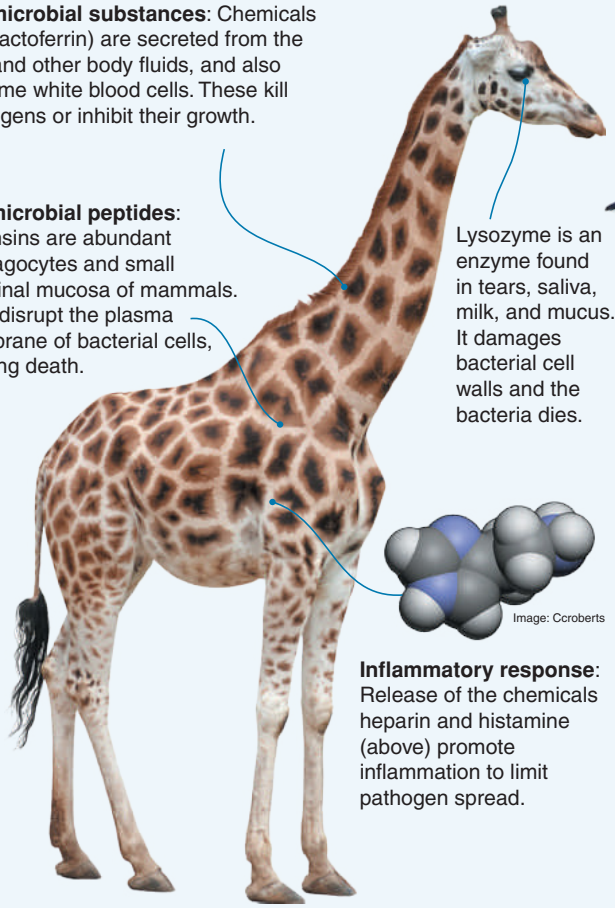
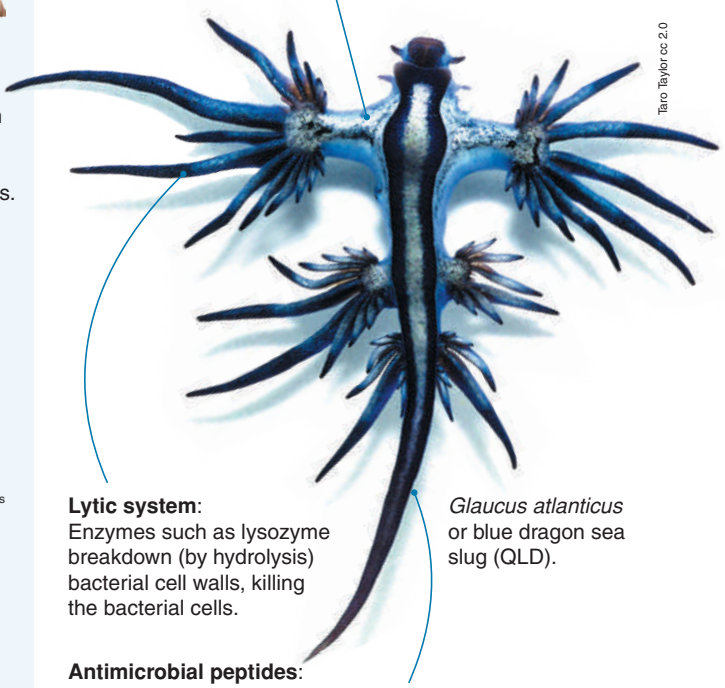


Image: Ccroberts

Inflammatory response: Release of the chemicals heparin and histamine (above) promote inflammation to limit pathogen spread.

Invertebrate innate defences

ProPO system: Microbial compounds induce the prophenoloxidase (proPO) defence system. proPO produces a cascade, and the final product, melanin, encases and kills the pathogen. The system is present in most invertebrates.



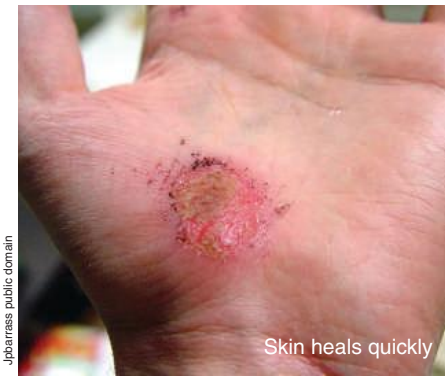
Taro Taylor cc 2.0

Lytic system: Enzymes such as lysozyme breakdown (by hydrolysis) bacterial cell walls, killing the bacterial cells.

Glaucus atlanticus or blue dragon sea slug (QLD).

Antimicrobial peptides: The antimicrobial peptide **defensin** binds to the cell membrane of pathogens, and causes damage by puncturing the membrane. The pathogen becomes 'leaky' and dies.

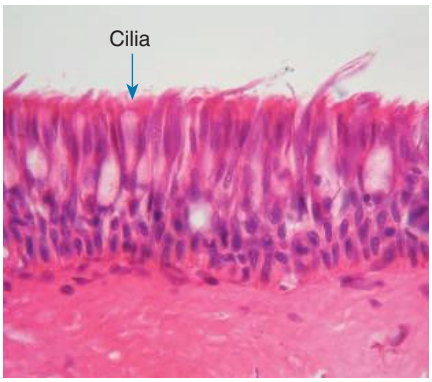
Physical defences in animals



Jpbarrass public domain

Skin heals quickly

Tears wash pathogens away



Physical barriers: Undamaged skin provides a waterproof, physical barrier to stop pathogens entering the body. Your skin is covered in microbes, but unless the skin is cut or grazed the microbes cannot cross the skin and enter your body. Even if your skin is damaged, it quickly begins to form a scab as it heals (above). This reduces pathogen entry into the body.

Mucous membranes and secretions: The eyes, mouth, nose, and urinary tract are entry points for pathogens. However, secretions (tears, saliva, and mucus) help keep pathogens out by washing them away. Some entry points are lined with mucous membranes. The cells of the mucous membrane secrete sticky mucus to trap pathogens and prevent them causing harm.

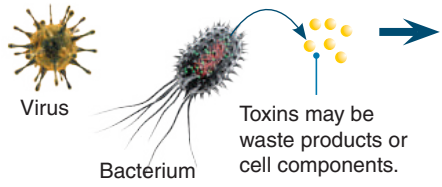
Mechanical defences: These physically remove microbes from areas where they might cause infection. Tiny, hair-like cilia move in a wave-like motion to carry mucus-trapped pathogens out of the body. This 'mucociliary escalator' moves trapped pathogens in the airways (above) away from the lungs, where the material can be expelled or swallowed and destroyed in the stomach.

ISBN: 978-1-98-856655-9

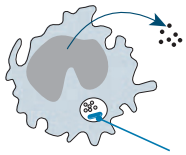
Responding to pathogens: fever

- White blood cells responding to pathogens release the protein interleukin-1. The release of interleukin-1 helps to reset the thermostat of the body to a higher level and also increases production of T cells which help activate other cells of the immune system. In the presence of certain viruses, cells also release the protein interferon, which interferes with viral replication.
- The higher body temperature caused by the presence of interluekin-1 helps intensify the effect of interferon and may also directly inhibit virus and bacteria development. Fever also increases heart rate so that white blood cells are delivered to sites of infection more rapidly.

1 The most frequent cause of fever is infection from bacteria (and their toxins) and viruses.



2 White blood cells responding to the pathogen release interleukin-1.

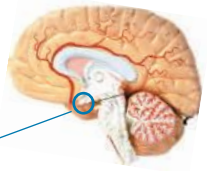


Macrophage releases **interleukin-1** into the bloodstream.

Macrophage digests bacterium in vacuole.

3 Interleukin-1 circulates to the brain and induces the hypothalamus to produce the lipid compound prostaglandin, which resets the 'thermostat' to a **higher temperature**, producing fever.

The hypothalamus controls the body's temperature setting.



4 The body responds to a higher temperature set point by constricting blood vessels, increasing metabolic rate, and shivering. These responses raise body temperature beyond the normal range of 36.2 – 37.2 °C.

5 Body temperature is maintained at the higher setting until the interleukin-1 has been eliminated. As the infection subsides, the thermostat is reset to 37°C. The person sweats to lose heat.

1. (a) Describe the advantage of having multiple (non-specific) defence responses: _____

   (b) Describe a disadvantage of having only general (non-specific) defence responses: _____

2. Compare and contrast the chemical defences of vertebrate and invertebrate animals: _____

3. What features of physical defences make them important as the first line of defence against pathogens?

4. Explain how fever occurs, and also its purpose: _____

ISBN: 978-1-98-856655-9

167 Chapter Review: Did You Get It?

1. Explain the importance of the MHC in the immune response: _____

2. The table below shows the response on selected Australian trees to infection by the pathogen *Phytophthora cinnamomi*.

Tree species	Number inoculated	Number dead	Percent mortality
Prickly tea tree (*Leptospermum continentale*)	15	4	
Prickly tea tree (*Leptospermum juniperinum*)	15	5	
Kangaroo acacia (*Acacia paradoxa*)	15	1	
Australia blackwood (*Acacia melanoxylon*)	15	1	
Varnish wattle (*Acacia verniciflua*)	15	8	
Spreading correa (*Correa decumbens*)	15	13	
Monarto mintbush (*Prostanthera eurybioides*)	15	7	
Flat pea (*Platylobium obtusangulum*)	6	6	

Response of selected South Australian native plant species to *Phytophthora cinnamomi*. K. H. Kueh, et al, 2012

(a) Complete the table above by calculating the % mortality of the trees:

(b) Which of the trees was the most susceptible to *Phytophthora*? _____

(c) What was the average mortality (%) of all the tree species? _____

3. Write letters A-F on the dotted line to match each term to its definition, as identified by its preceding letter code.

allergen _____

antigen _____

innate immune system _____

interferon _____

interleukin _____

pathogen _____

A Protein involved in the regulation of the immune response including inflammation and fever.

B Microorganism that causes disease.

C A substance causing an immune response in which the immune system overreacts to a normally harmless substance.

D A molecule that is recognised by the immune system as foreign.

E Generalised defence mechanisms against pathogens, e.g. physical barriers, secretions, inflammation, and phagocytosis.

F Protein released by animal cells in response to infection by a virus. It has the property of inhibiting viral replication.

4. Describe the responses of the innate immune system: _____

ISBN: 978-1-98-856655-9

CHAPTER 11

Immunity

Activity number

Key terms

active immunity
adaptive (=specific) immune response
antibody (=immunoglobulin)
antigen
artificially acquired immunity
B cell (B lymphocyte)
cellular (cell mediated) immunity
clonal selection
dendritic cell
humoral immunity
immunised
immunity
immunological memory
infection
leucocyte
lymph
lymph node
lymphocyte
macrophage
MHC
naturally acquired immunity
passive immunity
phagocyte
primary response
secondary response
T cell (T lymphocyte)
T helper cell
T killer cell
thymus
vaccination

Inquiry question: *How does the human immune system respond to exposure to a pathogen?*

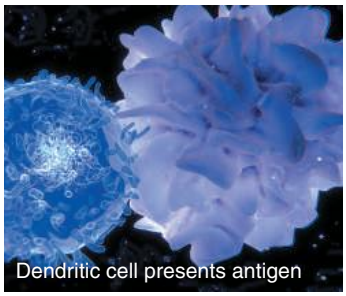
The innate immune response

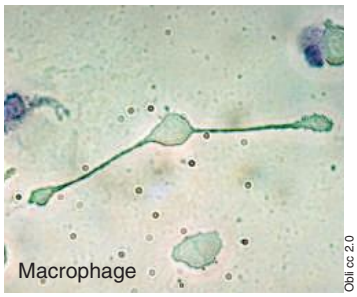
Key skills and knowledge

☐ 1 Describe the features and roles of the components of the innate (or non-specific) immune response to antigens, including the steps in inflammatory response. **168 169**

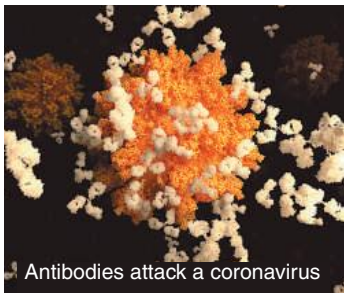
☐ 2 Describe the process of phagocytosis and explain the role of chemical markers and phagocyte receptors in phagocytosis. **170**

☐ 3 Describe the role of antigen-presenting cells, such as dendritic cells and macrophages, in initiating an immune response by processing and presenting antigens to lymphocytes. **171**


Dendritic cell presents antigen


Macrophage

Obli cc 2.0


Antibodies attack a coronavirus

The role of the lymphatic system

Key skills and knowledge

☐ 4 Explain the role of the lymphatic system in the immune response. Include reference to the role of lymph nodes as the site of antigen recognition by lymphocytes and the importance of the lymphatic system in transporting immune cells (e.g. dendritic cells) around the body. **172**

The adaptive immune response

Key skills and knowledge

☐ 5 Describe the features and roles of the components of the adaptive immune response (cellular and humoral immunity), including the role of specificity and memory. Contrast the adaptive immune response against both extracellular and intracellular threats. **173**

☐ 6 Recognise clonal selection and the basis of immunological memory. Explain how the immune system is able to respond to the large range of potential antigens. **174**

☐ 7 Describe the actions of B-lymphocytes (B cells) and their antibodies in humoral immunity. Include reference to antibody structure and how this relates to function. Describe some of the ways in which antibodies can inhibit infection. **173 175**

☐ 8 Describe the actions of T-lymphocytes in cellular immunity to include T helper and T cytotoxic (killer) cells. **173**

Natural and artificial immunity

Key skills and knowledge

☐ 9 Distinguish between naturally acquired and artificially acquired immunity. **176**

☐ 10 Describe active and passive strategies for acquiring immunity. Using examples, explain how both active and passive immunity can be acquired naturally or artificially. **176 177**

☐ 11 Describe primary and secondary responses to infection and explain how these responses form the basis of vaccination (immunisation) programmes. **176**

168 The Body's Defences: An Overview

Key Idea: The human body has a tiered system of defences that, together, provide resistance against disease.

The human body has a range of physical, chemical, and biological defences that provide **resistance** against pathogens. The first line of defence consists of external barriers to prevent pathogens entering the body. If this fails, a second line of defence targets any foreign bodies that enter. Lastly, the specific immune response provides a targeted third line of defence against the pathogen. The defence responses fall into two broad categories, the innate and the adaptive immune responses. The **innate** (or non-specific) response make up the first and second lines of defence. It protects against a broad range of non-specific pathogens. This response is present in all animals. It involves blood proteins (e.g. complement), inflammation, and phagocytic white blood cells. The **adaptive** (or specific) immune response is the third line of defence. It is specific to identified pathogens and is present only in vertebrates. It involves defence by specific T cells (cellular immunity) as well as antibodies, which neutralise foreign antigens (humoral immunity).

Most microorganisms find it difficult to get inside the body. If they succeed, they face a range of other defences that protect the body.

The natural populations of harmless microbes living on the skin and mucous membranes inhibit the growth of most pathogenic microbes.

Microorganisms are trapped in sticky mucus and expelled by cilia (tiny hairs that move in a wavelike fashion).

1st line of defence

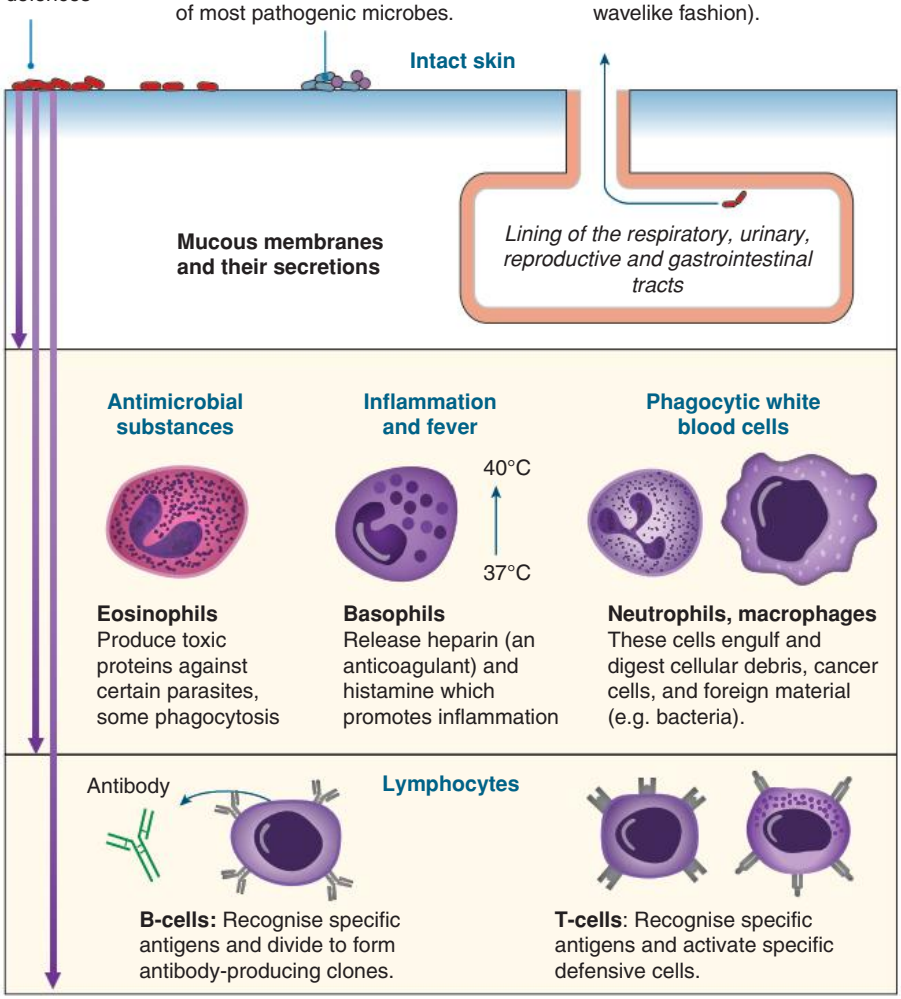
The skin provides a physical barrier to the entry of pathogens. Healthy skin is rarely penetrated by microorganisms. Its low pH is unfavourable to the growth of many bacteria and its chemical secretions (e.g. sebum, antimicrobial peptides) inhibit growth of bacteria and fungi. Tears, mucus, and saliva also help to wash bacteria away.

2nd line of defence

A range of defence mechanisms operate inside the body to inhibit or destroy pathogens. These responses react to the presence of any pathogen, regardless of which species it is. White blood cells are involved in most of these responses. The 2nd line of defence includes the complement system, whereby blood plasma proteins work together to bind pathogens and induce inflammation to help fight infection.

3rd line of defence

Once the pathogen has been identified by the immune system, **lymphocytes** (specialised white blood cells) launch a range of specific responses to the pathogen, including the production of defensive proteins called **antibodies**. Each type of antibody is produced by a B-cell clone and is specific against a particular antigen.



1. What are the differences between the innate and adaptive immune responses? _____

2. How does having a tiered defence help protect an organism from a pathogen? _____

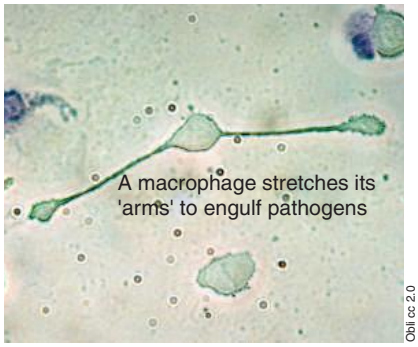
ISBN: 978-1-98-856655-9

169 The Innate Immune Response

Key Idea: The innate immune response provides a rapid response to contain and destroy pathogens. Inflammation is an important part of the response.

The innate immune system provides protection against a pathogen, even if it has never encountered it before. The innate response is very fast and provides general protection (it is not antigen specific), but does not provide long lasting immunity. Many different cells and processes are involved. The primary outcome is to destroy and remove the cause of infection. This is achieved by containing the infection through inflammation, and then recruitment of immune cells to destroy the pathogen. During this process a series of biochemical reactions (the complement system) are activated to destroy the pathogen and recruit immune cells to the site.

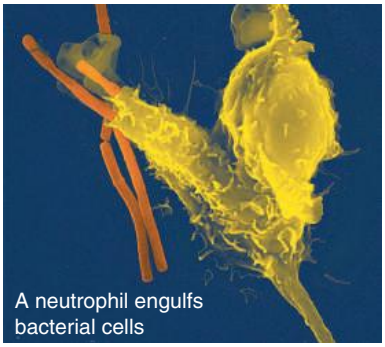
Phagocytic cells of the innate immune system



Obli cc 2.0

Macrophage

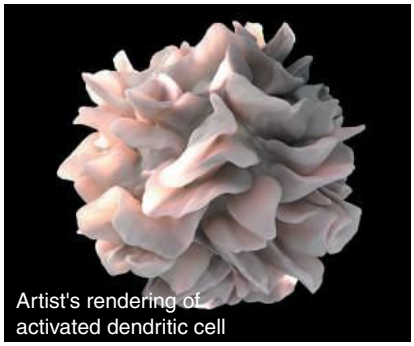
Macrophages are very large and are highly efficient phagocytes. They are found throughout the body and move using an amoeboid movement (above) to hunt down and destroy pathogens. Macrophages also have a role in recruiting other immune cells to an infection site.



Volker Brinkmann PLOS cc 2.5

Neutrophil

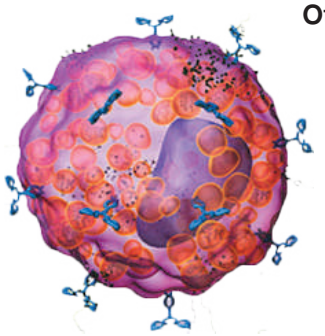
Neutrophils are the most abundant type of phagocyte and are usually the first cells to arrive at the site of an infection. They contain toxic substances that kill or inhibit the growth of bacteria and fungal pathogens. Neutrophils release cytokines which amplify the immune response and recruit other cells to the infection site.



Dendritic cell

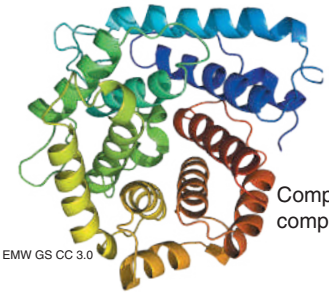
Dendritic cells are present in tissue that is in contact with the external environment (e.g. skin, and linings of the nose, lungs, and digestive tract). They act as messengers between the innate and adaptive immune system by presenting antigen materials to the T cells of the immune system.

Other cells and processes of the innate immune response



Mast cells

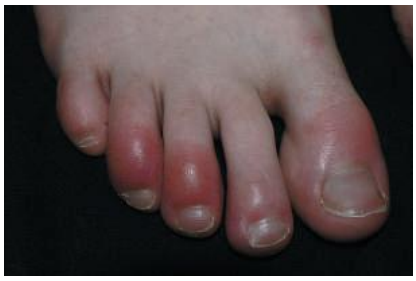
Mast cells contain a lot of histamine, a chemical involved in both inflammation and allergic responses. When activated, histamine is released from the mast cell causing the blood vessels to dilate and become leaky. The increased permeability allows phagocytes to reach the site of infection.



EMW GS CC 3.0

Complement proteins

The complement system comprises a number of different proteins. The proteins circulate as inactive precursors until they are activated. Complement proteins have three main roles: phagocytosis, attracting macrophages and neutrophils to the infection site, and rupturing the membranes of foreign cells.



The process of inflammation

The inflammatory process is a protective response to pathogen invasion. It has several functions: (1) to destroy the cause of the infection and remove it and its products from the body; (2) if this fails, to limit the effects on the body by confining the infection to a small area; (3) replacing or repairing tissue damaged by the infection.

1. Outline the role of the following phagocytes in the innate immune response:

(a) Macrophages: _____

_____

(b) Neutrophils: _____

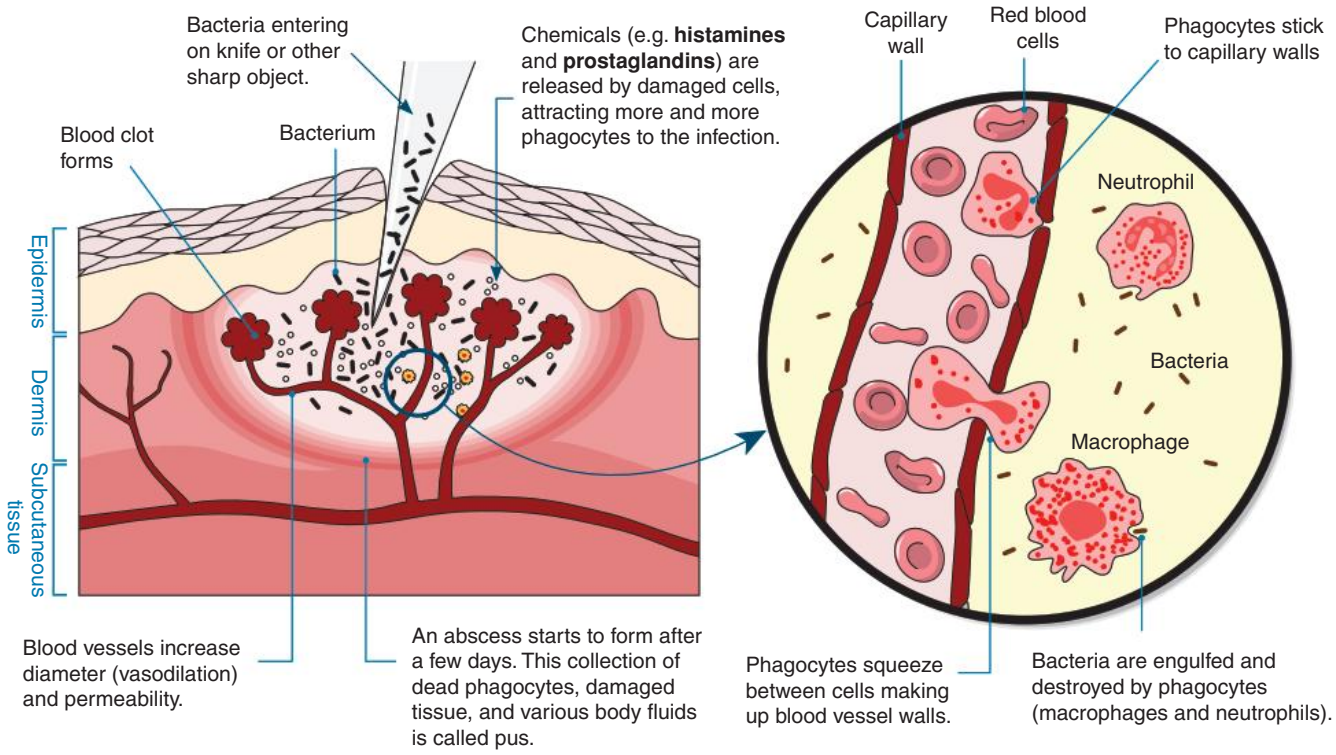
_____

(c) Dendritic cells: _____

_____

ISBN: 978-1-98-856655-9

The inflammatory response



Stages in the inflammatory response

Increased diameter and permeability of blood vessels
Blood vessels increase their diameter and permeability in the area of damage. This increases blood flow to the area and allows defensive substances to leak into tissue spaces.

Phagocyte migration and phagocytosis
Within one hour of injury, phagocytes appear on the scene. They squeeze between cells of blood vessel walls to reach the damaged area, where they destroy invading microbes.

Tissue repair
Functioning cells or supporting connective cells create new tissue to replace dead or damaged cells. Some tissue regenerates easily (skin) while others do not at all (cardiac muscle).

2. What role does the complement system play in immunity? _____

3. Outline the three stages of inflammation, and identify the beneficial role of each stage:

   (a) _____

   (b) _____

   (c) _____

4. What role do mast cells play in inflammation? _____

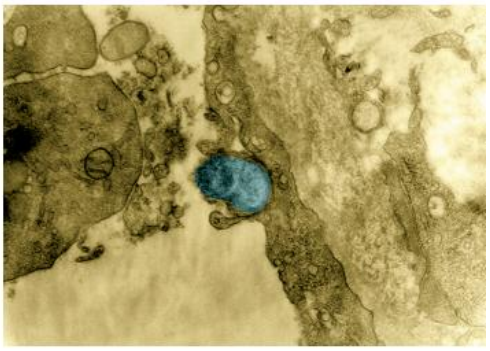
5. Why does pus form at the site of infection? _____

ISBN: 978-1-98-856655-9

170 Phagocytes and Phagocytosis

Key Idea: Phagocytes are mobile white blood cells that ingest and destroy extracellular foreign material and dead or dying cells.

Phagocytosis is the process by which a cell engulfs another cell or particle. Cells that do this are called phagocytes. All types of phagocytes (e.g. neutrophils, dendritic cells, and macrophages) are white blood cells. These specialised cells have receptors on their surfaces that can detect antigenic material, such as microbes. They then ingest the microbes and digest them, rendering them harmless. As well as destroying microbes, phagocytes also release substances called cytokines which help to coordinate the overall response to an infection. Macrophages and dendritic cells also play an important role in processing and presenting antigens from ingested microbes to other cells of the immune system.



CDC

Stages in phagocytosis and destruction of a pathogen

1. **Detection and interaction**
   Microbe coated in chemical markers is detected by the phagocyte, which attaches to it. Chemical markers coating the foreign material (e.g. a bacterial cell), mark it as a target for phagocytosis.

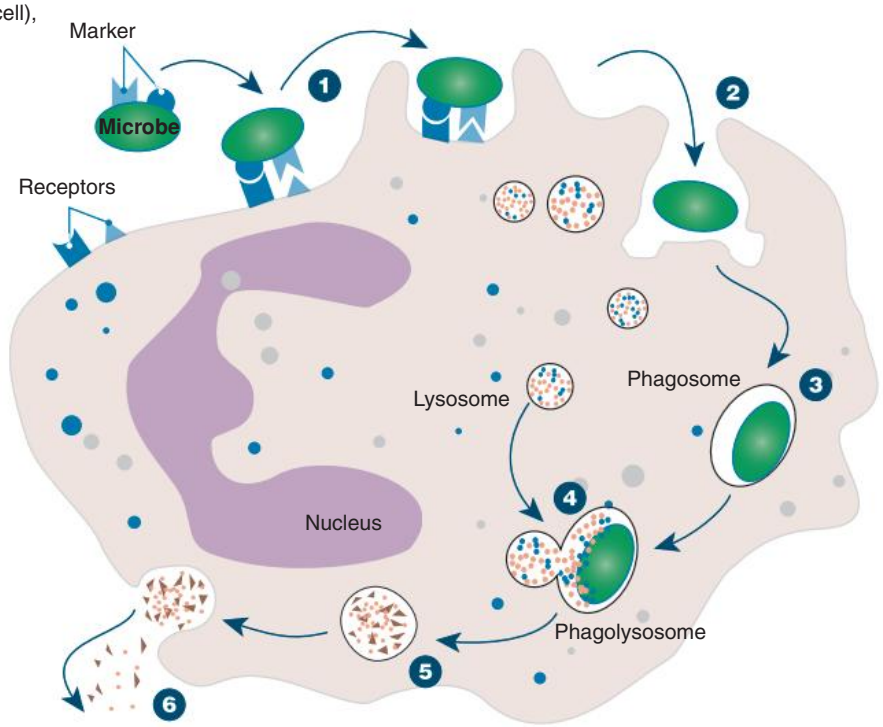
2. **Engulfment**
   The markers trigger engulfment of the microbe by the phagocyte. The microbe is taken in by endocytosis.

3. **Phagosome forms**
   A phagosome forms, enclosing the microbe in a membrane.

4. **Fusion with lysosome**
   Phagosome fuses with a lysosome containing digestive enzymes. The fusion forms a phagolysosome.

5. **Digestion**
   The microbe is broken down into its chemical constituents.

6. **Discharge**
   Indigestible material is discharged from the phagocyte.



1. Explain the role of chemical markers and phagocyte receptors in enhancing phagocytosis: _____

_____

_____

2. What is the purpose of phagocytosis, and how is it involved in internal defence? _____

_____

_____

_____

_____

3. Why do you think the foreign material has to be enclosed in a phagosome? _____

_____

ISBN: 978-1-98-856655-9

171 Processing Antigens

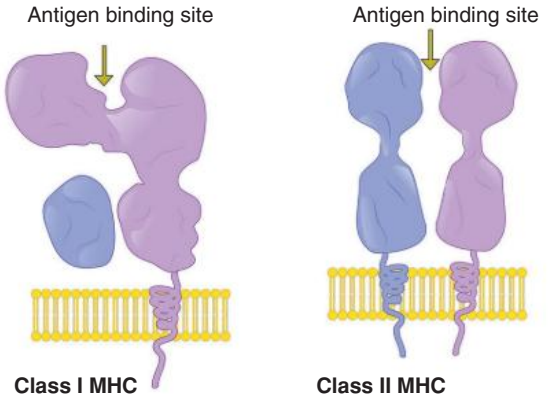
Key Idea: Antigen processing prepares and displays antigens for presentation to the T-cells of the immune system.

Antigen presenting cells (APCs) process and present antigens for recognition by T-cells. During antigen processing, the APC digests the foreign antigen into smaller peptide fragments. These fragments are then displayed on the surface of the APC by MHC receptors. The immune response evoked by the T-cells depends on which MHC receptor (MHCI or MHCII) is activated. Antigen presentation is necessary for T-cells to recognise infection or abnormal growth and activate other cells of the immune system. Dendritic cells, macrophages, and B-cells are APCs.

The role of MHC receptors

Recall there are two types of MHC receptors, class I and class II (right). Both have similar functions in that they display antigens on cell surfaces so that they can be recognised and processed by the T-cells of the immune system. T-cells can only recognise antigens if they are displayed by the MHC receptors. MHC receptors presenting no foreign antigens are ignored by T-cells, and are recognised as 'self'. Only MHC receptors with foreign antigens bound to them will attract T-cells and evoke an immune response.

The two classes of MHC receptors display different types of antigens. Class I MHC receptors display antigens from intracellular pathogens (e.g. viruses). Class II MHC receptors display antigens from pathogens that have been phagocytosed (e.g. bacteria).



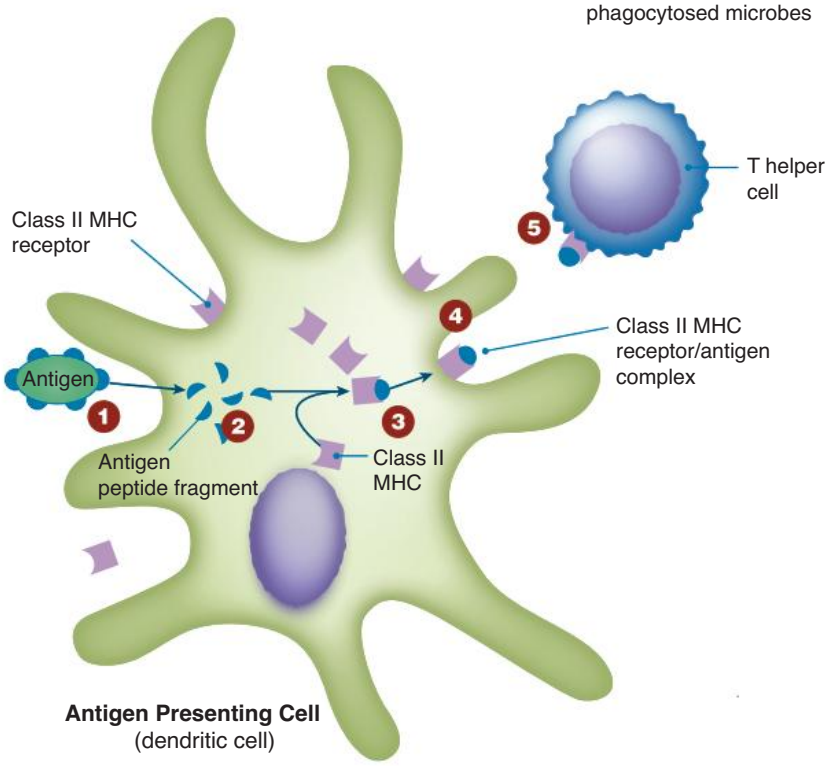
Class I MHC
Antigens from within the cell, e.g. viral proteins

Class II MHC
Antigens from outside the cell, e.g. proteins from phagocytosed microbes

An overview of antigen processing

The diagram on the right represents antigen processing of an extracellular peptide antigen via a class II MHC receptor.

1. An APC encounters an antigen.
2. The antigen is engulfed via phagocytosis and digested into short peptide fragments.
3. Class II MHC receptors bind the fragments and form a MHC-antigen complex.
4. The MHC-antigen complex is displayed on the surface of the APC.
5. A receptor on the T helper cell recognises the peptide as foreign. It binds and a series of events stimulate the adaptive immune response.



Antigen Presenting Cell
(dendritic cell)

1. What is the purpose of antigen processing? _____

2. Why do MHC receptors with no antigenic peptide bound not cause an immune response? _____

3. Describe the differences between class I and class II MHC receptors: _____

ISBN: 978-1-98-856655-9

172 The Lymphatic System and Immunity

Key Idea: The lymphatic system transports lymph, a fluid rich in white blood cells, throughout the body to attack antigens.

The lymphatic system is a network of tissues and organs that collects the tissue fluid leaked from the blood vessels and transports it to the heart. The lymphatic system has an important role in immunity because the fluid (lymph) transported by the lymphatic system is rich in infection-fighting white blood cells called lymphocytes. The thymus and red bone marrow are the primary organs of the lymphatic system, but it has many secondary components too.

Components of the lymphatic system

Tonsils
A collection of secondary lymphoid tissues in the throat. They provide defence against ingested or inhaled pathogens and produce activated B and T cells.

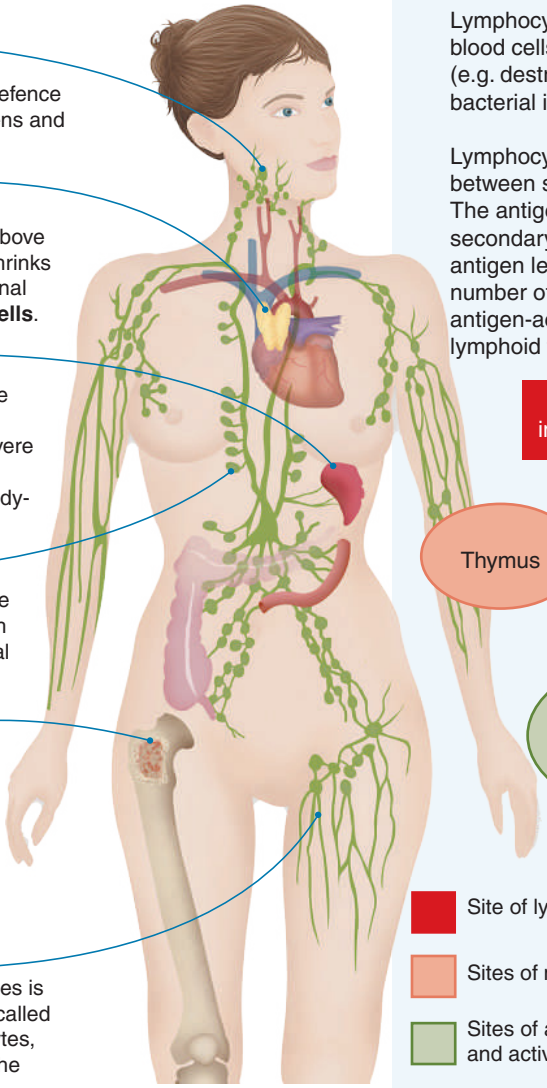
Thymus
A primary lymphoid organ located above the heart. It is large in infants and shrinks after puberty to a fraction of its original size. Important for maturation of **T-cells**.

Spleen
The largest mass of lymphatic tissue in the body. It stores and releases blood in case of demand (e.g. in severe bleeding), produces mature B-cells and antibodies, and removes antibody-coated antigenic material.

Lymph nodes
Ovoid masses of lymph tissue where lymphocytes are concentrated. Each node receives lymph through several incoming and outgoing vessels.

Red bone marrow
A primary lymphoid tissue where all the different kinds of blood cells (including white blood cells) are produced by cellular differentiation from stem cells. B cells also mature here.

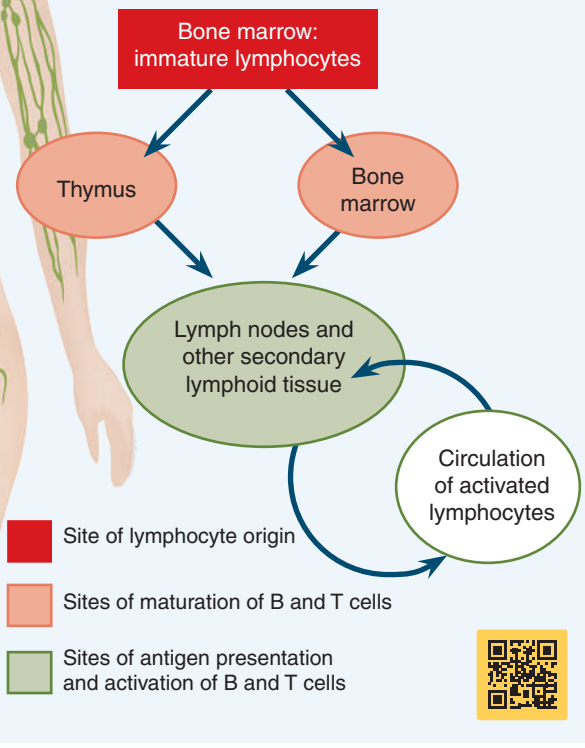
Lymphatic vessels
When the fluid leaking from capillaries is picked up by lymph capillaries, it is called **lymph**. The lymph, carrying leucocytes, flows in lymphatic vessels through the secondary lymphoid tissues.



The lymphatic system and immunity

Lymphocytes (T and B cells) are types of white blood cells. They are important in fighting infection (e.g. destroying the microbes causing viral or bacterial infections).

Lymphocytes in circulation are constantly moving between sites where antigens may be encountered. The antigens are presented to T cells in the secondary lymphoid tissues. Recognition of the antigen leads to activation and rapid increase in the number of both T and B cells. After several days, antigen-activated lymphocytes begin leaving the lymphoid tissue.



1. What is the general role of the lymphatic system in immunity? _____

2. (a) What is the role of lymph nodes in the immune response? _____

   (b) Why do you think lymph nodes become swollen when someone has an infection? _____

ISBN: 978-1-98-856655-9

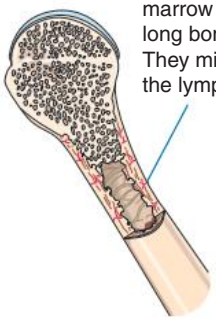
173 The Adaptive Immune Response

Key Idea: Antigens such as the cell walls of microbial cells, activate the immune system's B and T cells against specific pathogens when processed by antigen-presenting cells.

There are two main components of the adaptive immune system: the humoral and the cell-mediated responses. They work separately and together to protect against disease. The **humoral immune response** is associated with the serum (the non-cellular part of the blood) and involves the action of antibodies secreted by B cells (B lymphocytes). Antibodies are found in extracellular fluids including lymph, plasma, and mucus secretions and protect against viruses, and bacteria and their toxins. The **cell-mediated immune response** is associated with the production of specialised lymphocytes called **T cells**. Antigens are recognised by T cells only after antigen processing. The antigen is first engulfed by an antigen-presenting cell, which processes the antigen and presents it on its surface. T helper cells can then recognise the antigen and activate other cells of the immune system.

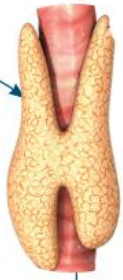
Lymphocyles and their functions

Bone marrow
B cells mature in the bone marrow in the shaft of the long bones (e.g. the femur). They migrate from here to the lymphatic organs.

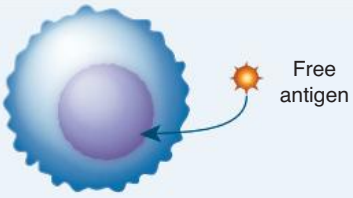


Stem cell
Stem cells in the bone marrow and fetal liver give rise to T cells and B cells.

Thymus gland
The thymus gland is located above the heart. It is large in infants but becomes smaller with age. Immature T cells move to the thymus to mature before migrating to other lymphatic organs.

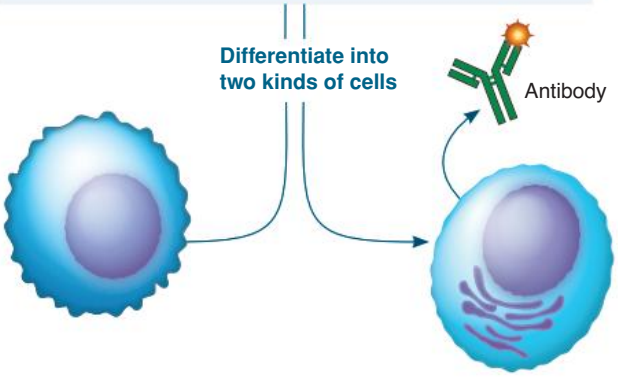


B CELLS



B cells recognise and bind antigens. Each B cell recognises one specific antigen. Helper T cells recognise specific antigens on B cell surfaces and induce their maturation and proliferation. A mature B cell may carry as many as 100,000 antigenic receptors embedded in its surface membrane. B cells defend against bacteria and viruses outside the cell and toxins produced by bacteria (free antigens).

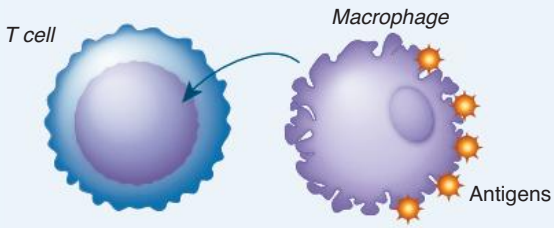
Differentiate into two kinds of cells



Memory cells
Some B cells differentiate into long-lived memory cells (see Clonal Selection). When these cells encounter the same antigen again (even years or decades later), they rapidly differentiate into antibody-producing plasma cells.

Plasma cells
When stimulated by an antigen (see Clonal Selection), some B cells differentiate into plasma cells, which secrete **antibodies** into the bloodstream. The antibodies then inactivate the circulating antigens.

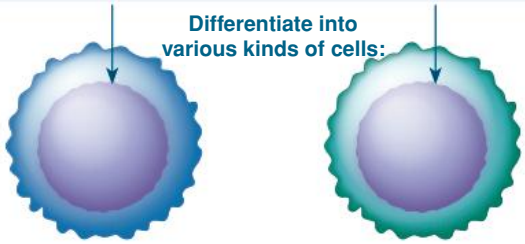
T CELLS



T cells respond only to antigen fragments that have been processed and presented by specialised antigen-presenting cells (dendritic cells and macrophages). They defend against:

- Intracellular bacteria and viruses
- Protozoa, fungi, flatworms, and roundworms
- Cancerous cells and transplanted foreign tissue

Differentiate into various kinds of cells:



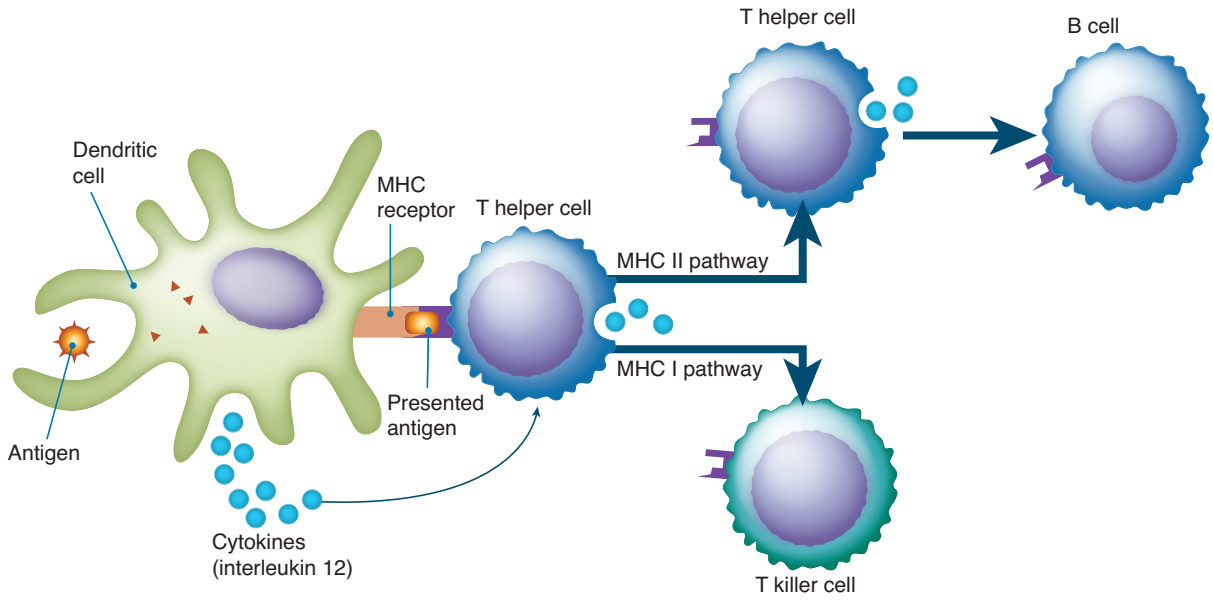
T helper cell activates T killer cells and other helper T cells. They are needed for B cell activation.

T killer cell destroys target cells on contact. Recognises tumour or virus-infected cells by their surface markers. They are also called cytotoxic T cells.

There are also other types of T cells:
T memory cells have encountered specific antigens before and can respond quickly and strongly when the same antigen is encountered again.
T regulator cells control the immune response by turning it off when no more antigen is present. They are important in the development of self tolerance.

ISBN: 978-1-98-856655-9

Dendritic cells stimulate the activation and proliferation of lymphocytes



- Dendritic cells (DC) are antigen-presenting cells. Immature DC originate in bone marrow and migrate through the body to lymph nodes. When a DC encounters an antigen, it presents it to a T helper cell, stimulating it to secrete chemicals called cytokines. Cytokines stimulate the activation and proliferation (rapid increase in number) of T cells, activating the immune system against that specific antigen. T helper cells go on to stimulate the production of antibody-producing B cells.
- Dendritic cells with MHC I receptors stimulate the production of T killer cells.
- Dendritic cells with MHC II receptors stimulate the production of T helper cells.

1. Where do B cells and T cells originate (before maturing)? _____

2. (a) Where do B cells mature? _____

   (b) Where do T cells mature? _____

3. Describe the nature and general action of the two major divisions in the immune system:

   (a) Humoral immune system: _____

   _____

   _____

   (b) Cell-mediated immune system: _____

   _____

   _____

4. Explain how an antigen causes the activation and proliferation of T cells and B cells, including the role of dendritic cells:

   _____

   _____

   _____

   _____

5. In what way do dendritic cells act as messengers between the innate and the adaptive immune systems?

   _____

   _____

6. Describe the function of each of the following cells in the immune system response:

   (a) T helper cells: _____

   (b) T killer cells: _____

ISBN: 978-1-98-856655-9

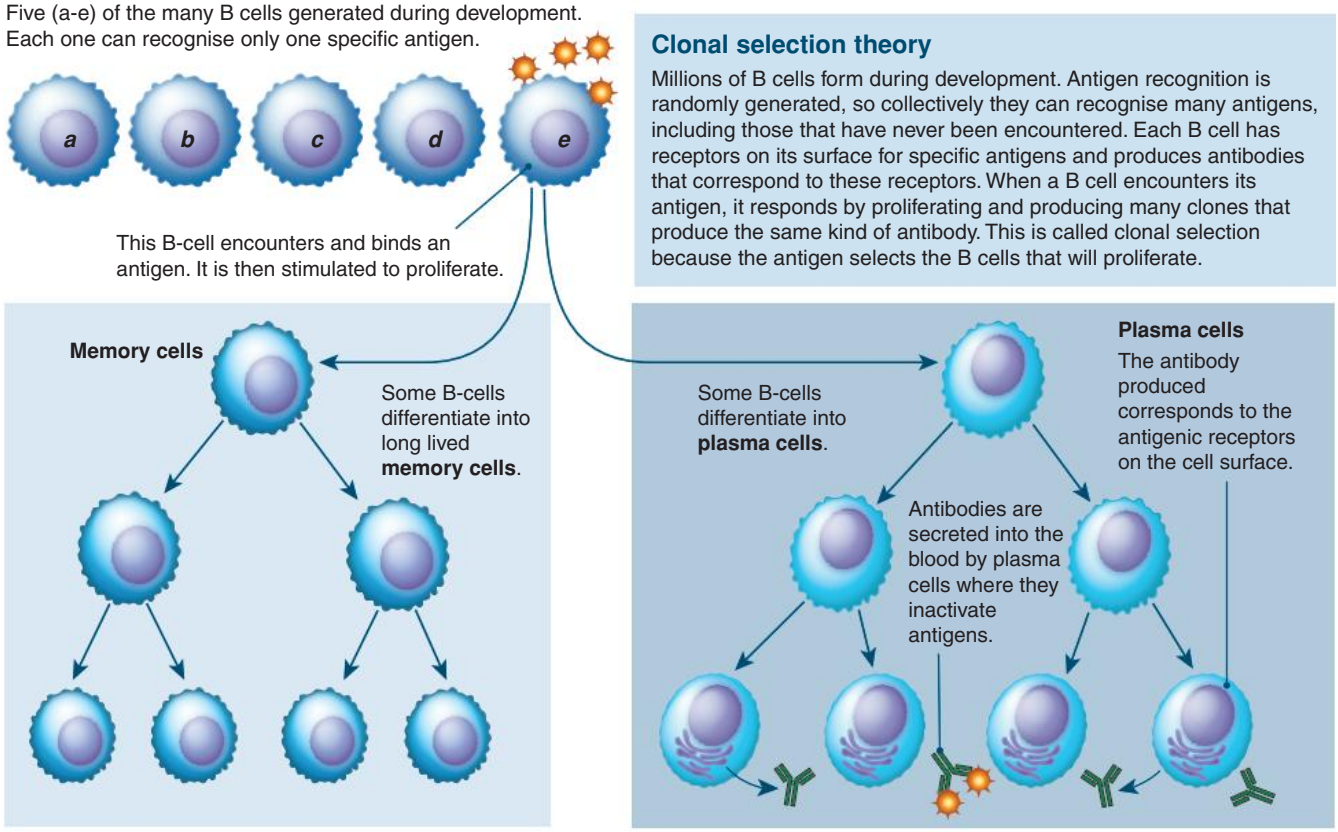
174 Clonal Selection

Key Idea: Clonal selection theory explains how lymphocytes can respond to a large and unpredictable range of antigens.

The **clonal selection theory** explains how the immune system can respond to the large and unpredictable range of potential antigens in the environment. The diagram below describes clonal selection after antigen exposure for B cells. In the same way, a T cell stimulated by a specific antigen will multiply and develop into different types of T cells. Clonal selection and differentiation of lymphocytes provide the basis for **immunological memory.**

Clonal selection theory

Millions of B cells form during development. Antigen recognition is randomly generated, so collectively they can recognise many antigens, including those that have never been encountered. Each B cell has receptors on its surface for specific antigens and produces antibodies that correspond to these receptors. When a B cell encounters its antigen, it responds by proliferating and producing many clones that produce the same kind of antibody. This is called clonal selection because the antigen selects the B cells that will proliferate.



Some B cells differentiate into long lived **memory cells**. These are retained in the lymph nodes to provide future immunity (**immunological memory**). If the antigen returns a second time, memory B cells react more quickly and vigorously than the first time the antigen appeared.

Plasma cells secrete antibodies specific to the antigen that stimulated their development. Each plasma cell lives for only a few days, but can produce about 2000 antibody molecules per second. During development, any B cells that react to the body's own antigens are destroyed in a process that leads to **self tolerance** (acceptance of the body's own tissues).

1. Describe how clonal selection results in the proliferation of one particular B cell clone: _____

2. (a) What is the function of the plasma cells in the immune system response? _____

   (b) What is the significance of B cells producing antibodies that correspond to (match) their antigenic receptors?

3. (a) Explain the basis of immunological memory: _____

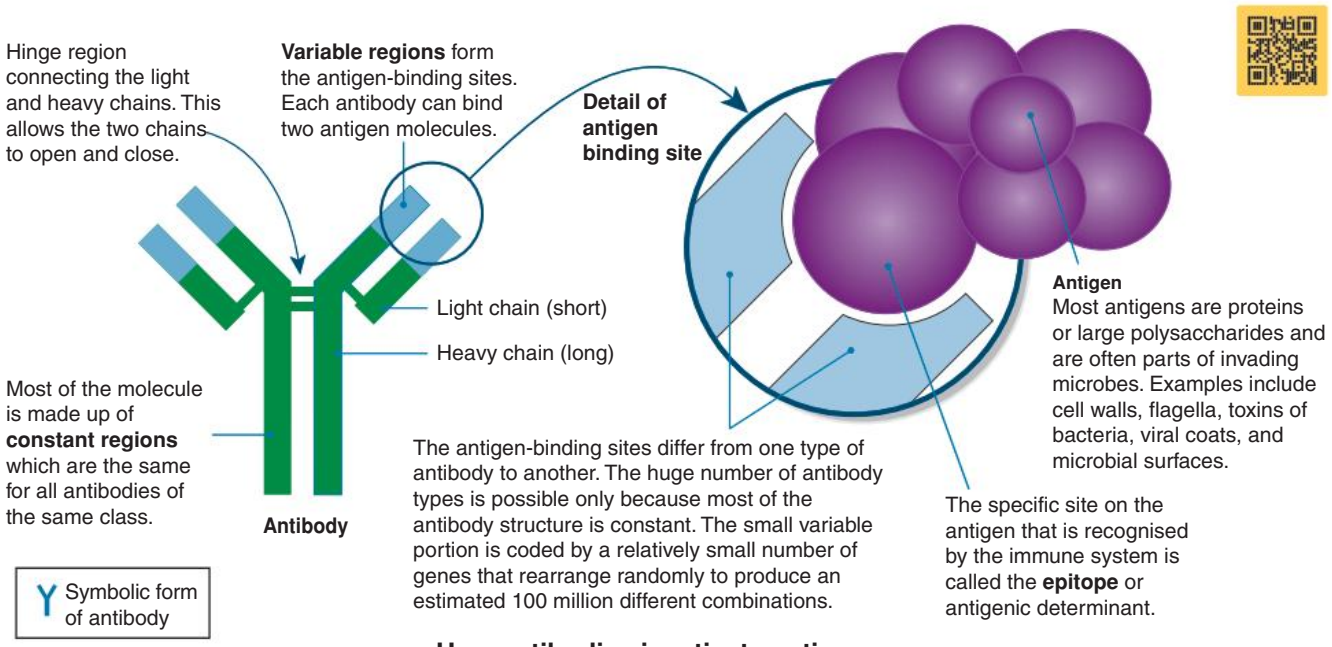
   (b) Why are B memory cells able to respond so rapidly to an encounter with an antigen long after an initial infection?

ISBN: 978-1-98-856655-9

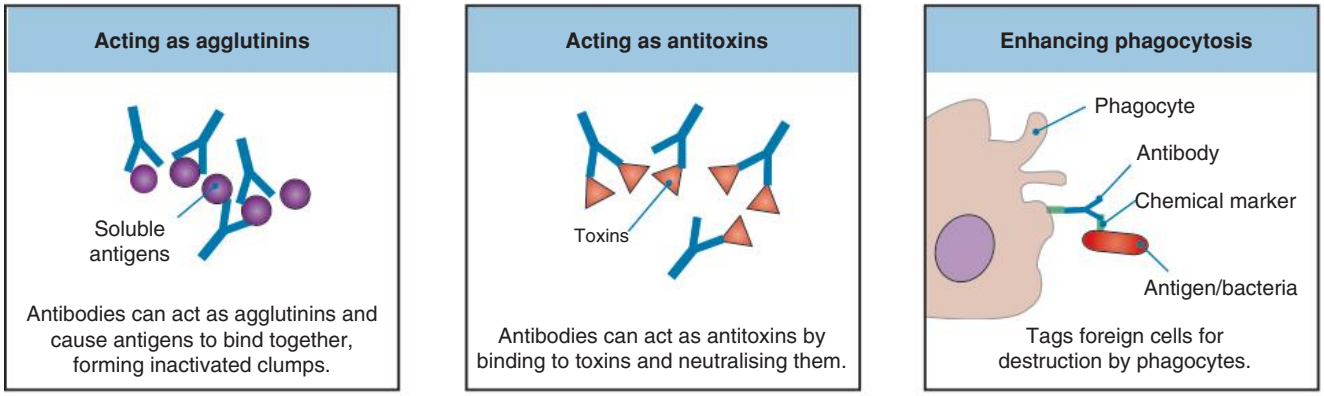
175 Antibodies

Key Idea: Antibodies are large, Y-shaped proteins made by plasma cells, which destroy specific antigens.

Antibodies and antigens play key roles in the response of the immune system. **Antigens** are foreign molecules which promote a specific immune response. Antigens include pathogenic microbes and their toxins, as well as substances such as pollen grains, blood cell surface molecules, and the surface proteins on transplanted tissues. **Antibodies** (or immunoglobulins) are proteins made in response to antigens. They are secreted from B cells into the plasma where they can recognise, bind to, and help destroy antigens. There are five classes of antibodies and each plays a different role in the immune response. Each type of antibody is specific to only one particular antigen.



How antibodies inactivate antigens



1. Describe the structure of an antibody, identifying the specific features of its structure that contribute to its function:

2. Explain how the following actions by antibodies enhance the immune system's ability to stop infections:

   (a) Acting as agglutinins:

   (b) Acting as antitoxins:

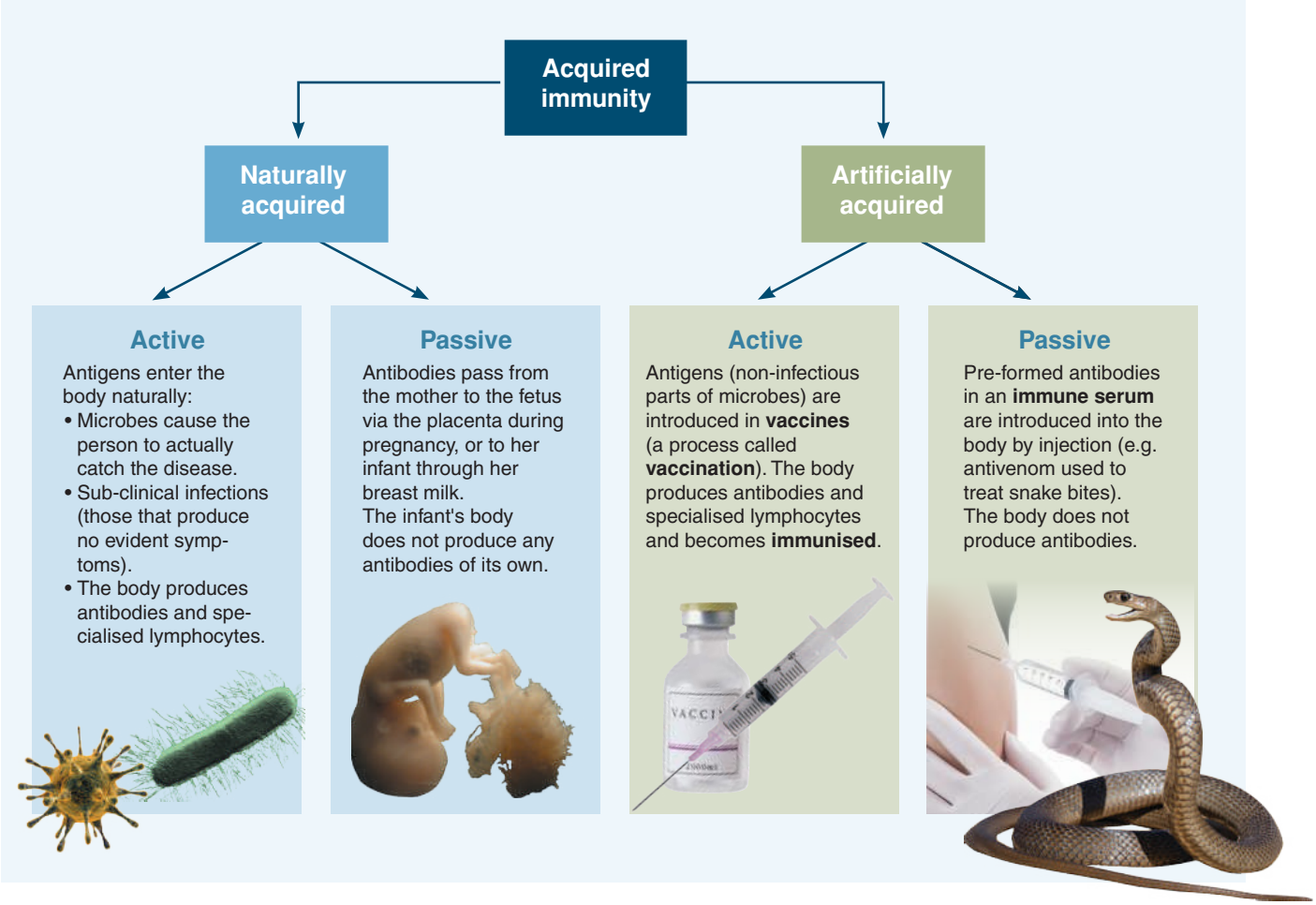
   (c) Tagging foreign cells with chemical markers:

ISBN: 978-1-98-856655-9

176 Acquired Immunity

Key Idea: Acquired immunity is a resistance to specific pathogens acquired over the life-time of an organism.

We are born with natural or **innate resistance** which provides non-specific immunity to certain illnesses. In contrast, **acquired immunity** is protection developed over time to specific antigens. **Active immunity** develops after the immune system responds to being exposed to microbes or foreign substances. **Passive immunity** is acquired from gaining pre-formed antibodies without exposure to the antigen. Immunity can be naturally acquired, through natural exposure to microbes, or artificially acquired as a result of medical treatment (below).



1. (a) What is meant by passive immunity? _____

_____

(b) Distinguish between naturally and artificially acquired passive immunity, and give an example of each: _____

_____

_____

_____

2. (a) Why does a newborn baby need to have received a supply of maternal antibodies prior to birth? _____

_____

_____

_____

(b) Why is this supply supplemented by antibodies in breast milk? _____

_____

3. (a) What is active immunity? _____

_____

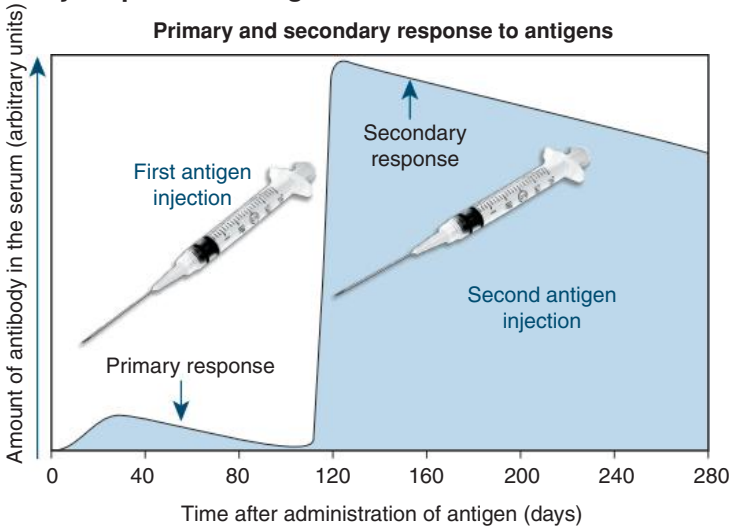
ISBN: 978-1-98-856655-9

Primary and secondary response to antigens

When the B cells encounter antigens and produce antibodies, the body develops active immunity against that antigen.

The initial response to antigenic stimulation, caused by the sudden increase in B cell clones, is called the primary response. Antibody levels as a result of the primary response, peak a few weeks after the response begins and then decline. However, because the immune system develops an immunological memory of that antigen, it responds much more quickly and strongly when presented with the same antigen subsequently (the secondary response).

This forms the basis of immunisation programmes, where one or more booster shots are provided following the initial vaccination.

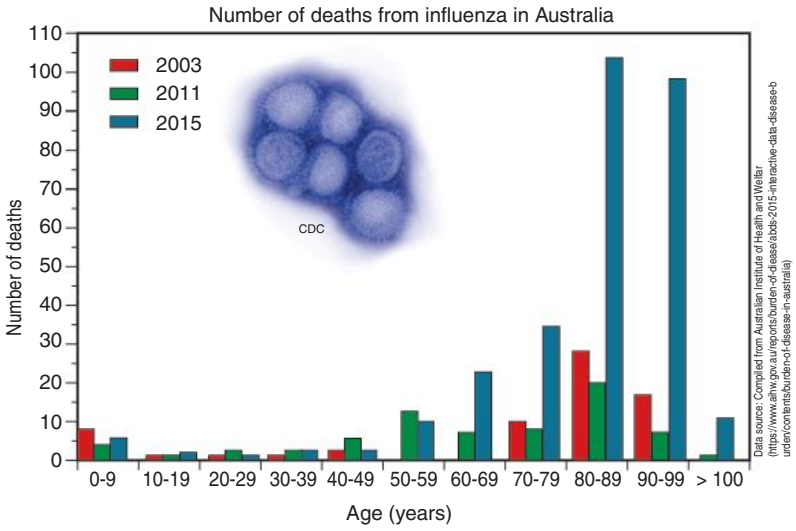


(b) Distinguish between naturally and artificially acquired active immunity, and give an example of each: _____

4. (a) Describe two differences between the primary and secondary responses to presentation of an antigen: _____

(b) Why is the secondary response so different from the primary response? _____

5. Some diseases do not affect all members of a population equally. Socioeconomic factors, age, sex, ethnicity, and where someone lives, can influence how a disease affects a particular individual or population. The data (right) shows deaths from influenza (flu) in Australia, by age, from three different years.



(a) Do you think the data shows an age related effect for influenza deaths? Explain your reasoning:

(b) Suggest what could be done to help reduce the number of influenza deaths: _____

ISBN: 978-1-98-856655-9

177 Chapter Review: Did You Get It?

1. Identify each of the following components of, or processes in, the adaptive immune system, by the description provided:

   (a) The process by which a lymphocyte is stimulated to respond to a specific antigen: _____

   (b) This cell type secretes antibodies specific to a particular antigen: _____

   (c) This cell type processes and presents antigens to T helper cells: _____

   (d) These T cells destroy target cells on contact: _____

   (e) These cells are responsible for immunological memory: _____

   (f) This class of proteins is made by B cells and destroy specific antigens: _____

   (g) These chemicals activate T cells and cause them to multiply rapidly: _____

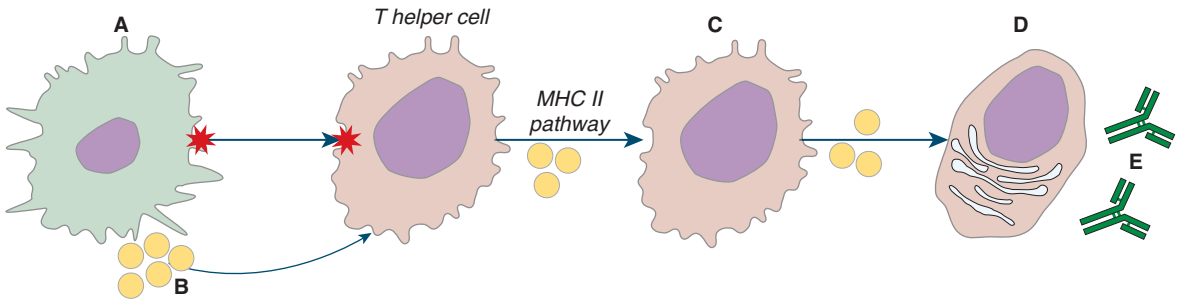
   (h) This immune system organ synthesises antibodies and removes antibody-coated material: _____

   (i) T cells mature in this organ: _____

   (j) Immunity developed over time to specific antigens: _____

2. Contrast the innate and the adaptive immune responses with reference to the basic action and the cells involved:

3. The diagram below shows a model of part of the adaptive immune system reaction when an antigen is encountered.



   (a) What type of cell is A and what is its role? _____

   (b) Identify the structures labelled B and state their role in this part of the process: _____

   (c) What type of cell is C? _____

   (d) What type of cell is D? _____

   (e) Identify the structures labelled E and state their role: _____

ISBN: 978-1-98-856655-9

CHAPTER 12

Prevention, Treatment, Control

Key terms
antibiotic
biosecurity
epidemic
incidence
herd immunity
outbreak
pandemic
prevalence
quarantine
vaccination
vaccine

Activity number

Inquiry question: *How can the spread of infectious diseases be controlled?*

Controlling the spread of disease

Key skills and knowledge

Investigating disease in populations

Key skills and knowledge

178 Containing the Spread of Disease

Key Idea: Preventing the entry and spread of pathogens is important in protecting a country's population and industries from infectious diseases.

Many factors can influence the spread of disease, including the political and social climate, diet, general health, and access to medical care. Human intervention, modification of behaviour, and vaccination, can reduce the transmission rate of some diseases and inhibit their spread. Global air travel and international trade in commodities has increased the risk of diseases affecting humans, livestock, and crops spreading rapidly between countries. Australia is fortunate because its geographical isolation has helped to prevent the spread of disease from other parts of the world. Even so, pandemics such as H1N1 swine flu and Covid-19 still reached Australia.

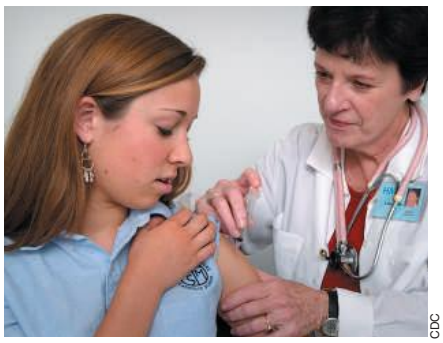
Transmission of disease can be reduced by adopting 'safe' behaviours. Examples include the use of condoms to reduce the spread of STIs, isolation of people already infected, or establishing quarantine procedures for people who have been exposed to infection.

The environment can be made less suitable for the growth and transmission of pathogens. For example, spraying drainage ditches and draining swamps eliminates breeding habitats for mosquitoes carrying diseases such as Zika virus and malaria.

Disinfectants and sterilisation techniques, e.g. autoclaving (above), destroy pathogenic microbes before they have the opportunity to infect. The use of these techniques in medicine has significantly reduced post-operative infections and associated deaths.

The development of effective sanitation, sewage treatment, and treatment of drinking water has virtually eliminated dangerous waterborne diseases from developed countries. These practices disrupt the normal infection cycle of pathogens such as cholera and giardia.

Appropriate personal hygiene practices reduce the risk of infection and transmission. Soap may not destroy the pathogens but washing will dilute and remove them from the skin. Although popular, antibacterial soaps encourage the development of strains resistant to antimicrobial drugs.



CDC

Vaccination schedules form part of public health programmes. Vaccination is one of the most effective ways of preventing transmission of contagious diseases. If most of the population is immunised, herd immunity limits outbreaks to sporadic cases and prevents epidemics.

1. (a) Identify three ways in which the environment can be made less suitable for establishment and transmission of diseases:

_____

_____

_____

(b) How is disease transmission reduced in medical care situations? _____

_____

(c) Why is sanitation important in preventing the spread of disease? _____

_____

_____

2. Why is reducing the prevalence of disease preferable to trying to contain an outbreak? _____

_____

_____

ISBN: 978-1-98-856655-9

Investigating the use of domestic pesticides to prevent disease

- Pesticides (fungicides, insecticides, etc) play an important role in preventing disease spread. In particular, insects such as mosquitoes play a major role in spreading diseases. Malaria is transmitted by the *Anopheles* mosquito and is a major disease threat in tropical parts of the world. In Australia, mosquito borne diseases include Ross River virus, Barmah Forest virus, and Murray Valley encephalitis.
- Protecting yourself from these diseases can involve actions such as using insecticides. In the home, domestic sprays can help remove insect pests that spread disease. Repellents, such as using DEET, prevent biting insects reaching the skin.

3. In groups of three, investigate the use of domestically available pesticides in the prevention of the spread of disease. The **BIOZONE Resource Hub** has some useful links:

    - Each member should investigate a different aspect of pesticide use, such as the kind of pesticides used, their effectiveness, any side effects on people etc.
    - Each member should report back to the group with their findings and then write their own conclusions and discussion based on the research presented.
    - What are some advantages and disadvantages of these domestically used pesticides in disease control?

Investigating the use of genetic engineering in disease prevention

- Recall that CRISPR has become an important tool in genetic engineering and that it can be used to prevent the spread of disease carrying organisms and so limit the spread of disease itself.
- Proposals for the use of genetic engineering in the prevention of disease include modifying genes to prevent viral entry into cells. This has already happened in humans. In 2018, Chinese scientist He Jiankui announced that he had edited the DNA of twin babies to remove the CCR5 gene which produces a cell surface receptor that allows the HIV virus to enter the cell.

4. Research this episode of genetic editing for prevention of infectious disease. The **BIOZONE Resource Hub** has some useful links.

    - How was it done, why, and what were the repercussions?
    - Discuss the ethics of this technology. Do you agree with the use of the technology for these reasons?
    - Discuss your opinion with your class mates. Record your ideas below:

ISBN: 978-1-98-856655-9

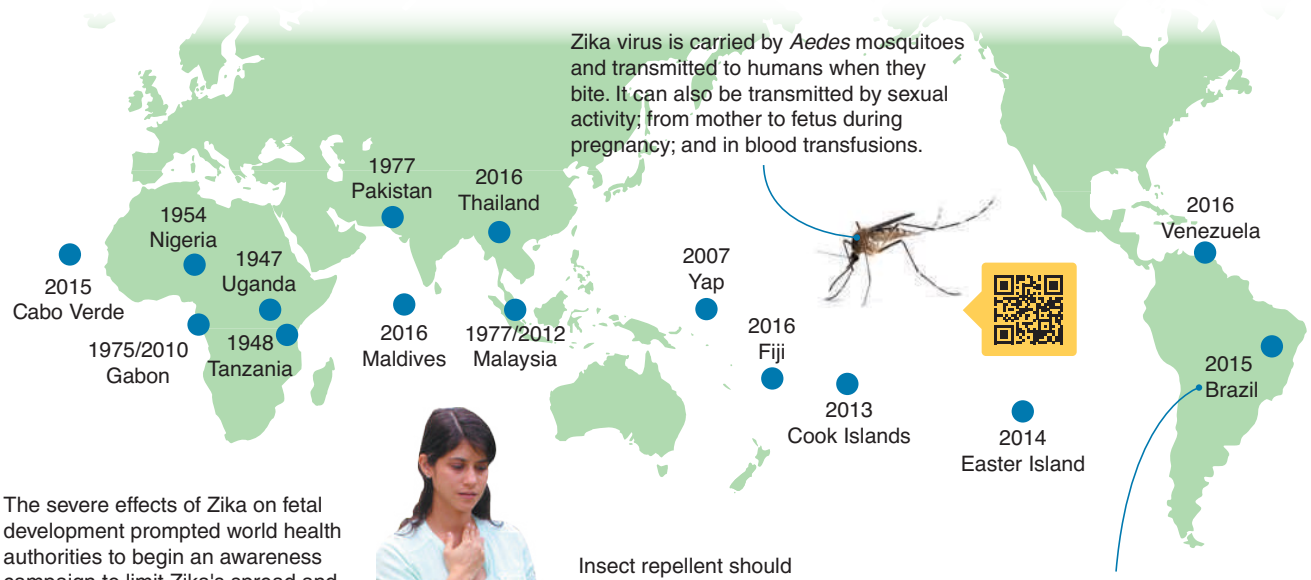
179 Patterns of Disease

Key Idea: Studying the prevalence and spread of a disease gives insights into its origins and how to combat it.

Diseases present at constant low levels in a population or region are known as **endemic** diseases. Occasionally, there may be a sudden increase in the **prevalence** of a particular disease. On a local level this is known as an **outbreak**. When an infectious disease spreads rapidly through a nation and affects large numbers of people it is called an **epidemic**. On rare occasions a new kind of disease will appear and spread to other countries. The rapid spread of a disease throughout the world is a **pandemic**. Examples of pandemic diseases include HIV/AIDS, influenza, Covid-19, and Zika virus.

Zika virus: An example of global disease spread and its containment

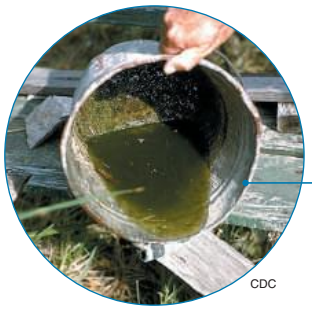
- Zika virus was first isolated from the Zika Forest in Uganda in 1947. Since then, it has spread slowly across the globe, with outbreaks in the Americas in 2015 and 2016. Zika causes a mild fever and rash that is not usually serious in adults. However, in the last few years, infection of pregnant women by Zika has been linked to microcephaly (small head and brain) in their newborns.



The severe effects of Zika on fetal development prompted world health authorities to begin an awareness campaign to limit Zika's spread and reduce the risk of people contracting it. The campaign focussed on prevention, and included travel advisories in unaffected countries, as well as awareness campaigns in affected countries.

Insect repellent should be used, particularly if wearing clothing that exposes the skin.

People are advised to wear long sleeves and pants to prevent mosquito bites.



Reducing areas where water can stagnate reduces mosquito breeding sites.

Zika virus became an important international concern in 2015 and 2016 in the lead up to, and during, the 2016 Rio de Janeiro Olympics. Concerns focussed on the movement of spectators, tourists, and athletes, and the spread of the disease around the globe as they returned home after the events.

1. (a) In which general direction has Zika virus spread across the globe? _____

   (b) Describe the area that Zika virus appears to be generally confined to and explain this: _____

   _____

2. How is Zika virus transmitted? _____

   _____

3. Describe how the spread of Zika virus can be reduced: _____

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

Seasonal patterns

Some diseases are seasonal, showing patterns of increased or decreased prevalence at specific times of the year. Influenza (the flu) commonly becomes more prevalent in the winter of both the Southern and Northern Hemispheres. Ross River fever becomes more common during the summer/autumn rainy season (January to March). The seasonal patterns of these diseases allow for simple predictions of when most cases will occur. For example, health authorities prepare for increased influenza cases in winter by offering the latest vaccines.

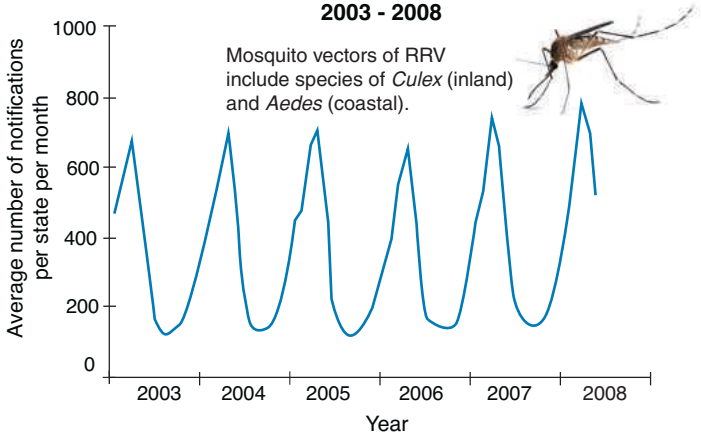
Ross River virus

Ross River virus (RRV), which causes Ross River fever, is endemic to Australia, Papua New Guinea, and several other islands of the South Pacific. RRV is transmitted by mosquitoes and may have natural reservoirs in native Australian mammals. In Australia, most RRV infections occur in the wetter, tropical areas of QLD, WA, and NT. There have been several large outbreaks since the first noted outbreak in 1928. The largest outbreak occurred in 1979-80 across the Western Pacific, affecting 60,000 people.

Ross River fever

Symptoms of Ross River fever include fever, rash, and arthritic-like symptoms causing extreme pain in various joints. In most cases, the disease lasts about a month but can reoccur over a period of years. Currently a blood test is the only way to confirm Ross River fever and there is no vaccine.

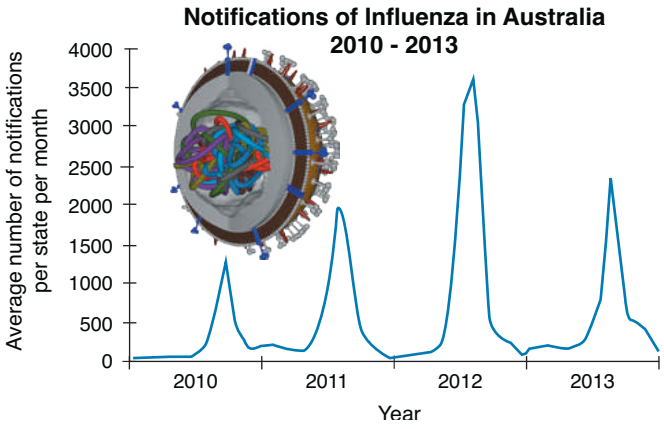
Notifications of Ross River virus in Australia, 2003 - 2008



Influenza

Although it is often looked upon as simply a bad cold, influenza (commonly called 'the flu') has arguably caused more human deaths than any other disease throughout history. Influenza occurs in seasonal epidemics, usually infecting around five million people globally, and causing the death of between 250,000 and 500,000 people annually. The influenza virus continually changes in two ways. **Antigenic drift** involves small cumulative genetic changes over time. Occasionally, the virus will also undergo **antigenic shift**, producing an entirely new strain that causes a pandemic. The latest of these is the H1N1/09 strain, commonly referred to as swine flu. This strain caused around 14,000 deaths globally. Compare this to the Spanish flu pandemic of 1918-1920, during which possibly up to 100 million people died globally (more than all deaths during World War I, and perhaps as many as World War II).

Notifications of Influenza in Australia 2010 - 2013



4. (a) How is the Ross River virus transmitted to people? _____

   (b) Suggest why cases of Ross River fever are more prevalent in January to March: _____

   _____

   _____

   (c) There was a large mosquito outbreak in 2013 in Newcastle, NSW. Predict the effect of this on the prevalence of Ross River fever:

   _____

5. (a) At what time of year is the influenza virus most prevalent? _____

   (b) Why must people receive the flu vaccine every year if they want to remain protected against influenza?

   _____

   _____

6. Why is it useful to track the incidence of diseases such as Ross River fever and influenza? _____

   _____

   _____

ISBN: 978-1-98-856655-9

180 The Effectiveness of Hand Washing

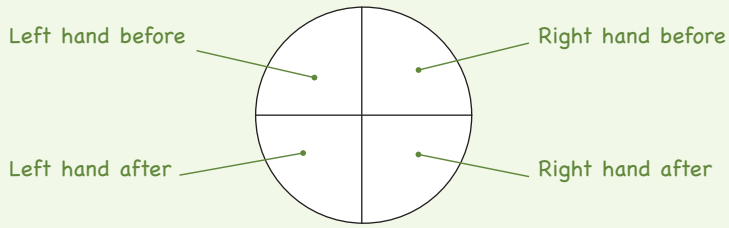
Key Idea: Hand washing is one of the most effective ways of preventing the spread of disease.

We, as humans, spend much of our time manipulating objects with our hands, so it follows that our hands are covered with the microorganisms found in our environment. These microbes can then be easily transferred by touch to our mouths, such as when eating, or to other people, such as when we hand them an object. Hand washing after contact with potentially contaminated material reduces the chance of transmitting microbes to our internal environment or to others. In the practical below you will obtain data on the effectiveness of handwashing.

Investigation 12.1 Investigating the effectiveness of handwashing

See appendix for equipment list.

1. The class will be divided into thirds. One third will wash their hands with warm water. One third will wash their hands with soap and warm water and one third will use hand sanitiser. Your teacher will place you into one of these groups. **Do not wash your hands until step 5!**
2. Each person in the group should take a nutrient agar plate and use a marker pen to label the edge of the lid of the plate with name, the incubation temperature (e.g. 30°C), and which group you are in.
3. Then use the marker pen to divide the plate lid into quarters and label them as shown below:



4. Open the lid and press the tips of your index and middle fingers from your left hand in the 'Left hand before' quarter. Hold them there for 5 seconds. Then press the tips of your index and middle fingers from your right hand in the 'Right hand before' quarter. Hold them there for 5 seconds. Close the lid.
5. Now, wash your hands using the regime assigned to your group (water, soap and water, hand sanitiser). Dry your hands, if necessary, with a clean paper towel.
6. Open the lid of the agar plate again and press the tips of your index and middle fingers from your left hand in the 'Left hand after' quarter. Hold them there for 5 seconds. Then press the tips of your index and middle fingers from your right hand in the 'Right hand after' quarter. Hold them there for 5 seconds. Close the lid and seal it with clear tape.
7. Incubate the plate at your chosen incubation temperature, lid down, for 24 hours.
8. Retrieve the agar plates and observe the four different quarters. Count and record the number of bacterial colonies on the plate in each half (before and after). Do this for all the plates in your assigned group. If you only have a small number in your group, just enter the data you have. Calculate the mean number of the colonies before and after (below).
9. Compare your means with means from the other groups in the class.

1. (a)

Your technique: _____	Plate number										Mean
Number of colonies before washing hands											
Number of colonies after washing hands											

(b) Handwashing technique: _____ Mean colonies before: _____ Mean colonies after: _____

(c) Handwashing technique: _____ Mean colonies before: _____ Mean colonies after: _____

2. Which technique appears to have the greater ability to remove bacteria from your hands? Explain why:

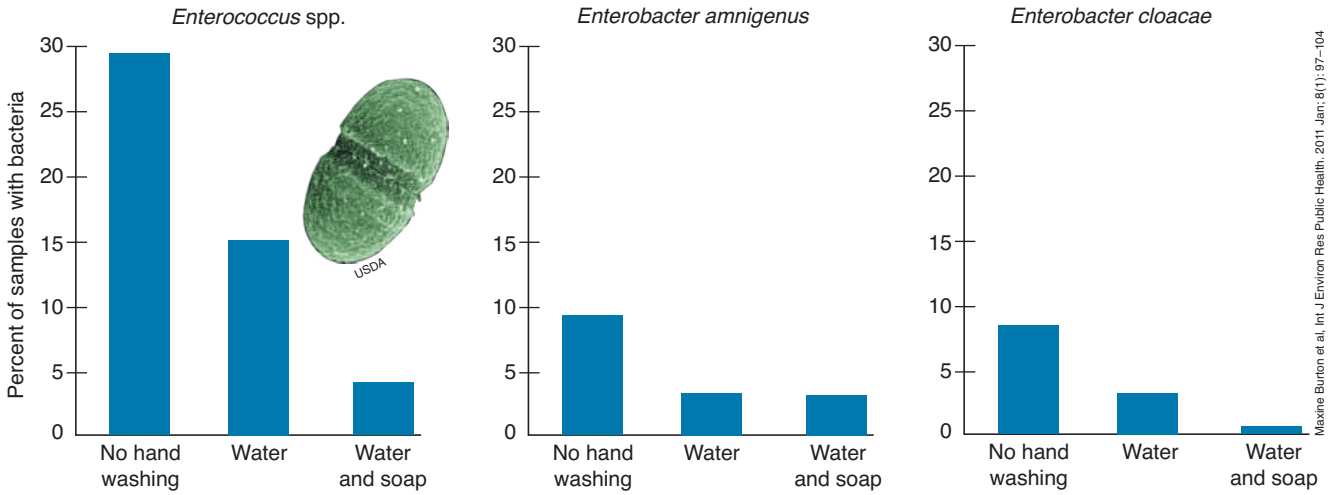
_____

_____

ISBN: 978-1-98-856655-9

Testing the effectiveness of hand washing

- A 2011 study performed the following experiment on the effectiveness of hand washing.
- Twenty volunteers deliberately contaminated their hands by contact with hard surfaces such as hand rails and door knobs in public areas.
- They were then randomly allocated to one of three groups: no hand washing, hand washing with tap water, or handwashing with soap and tap water. No instructions were given as to how to hand wash or for what length of time. Volunteers simply washed their hands as they would normally.
- Swabs were then taken from the volunteers' hands and transferred to agar plates. These were incubated at 35°C for 48 hours. This procedure was carried out 24 times for each volunteer for a total of 480 samples.
- The results are shown below. The bacteria found all occur in the intestines of animals (collectively called faecal coliforms).



Questions 3-5 are with reference to the study above:

3. (a) Was hand washing an effective way to remove bacteria from the hands? _____

   (b) Which was the most effective method of removing bacteria from the hands? _____

4. Which bacterium was most common on the hands? _____

5. Why do you think the researchers gave no handwashing instructions to the volunteers?_____

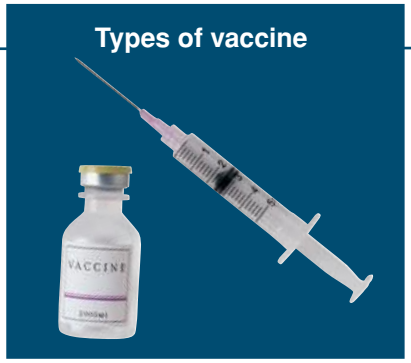
6. Use your data and the experiment above to explain why hand washing is an important part of controlling the spread of disease, especially in an epidemic or pandemic situation (e.g. the Covid-19 pandemic):

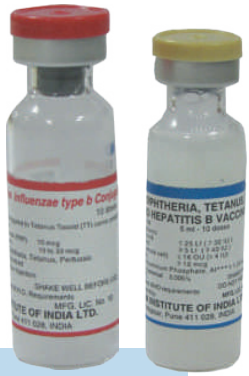
ISBN: 978-1-98-856655-9

181 Vaccines and Vaccination

Key Idea: A vaccine is a suspension of antigens that is deliberately introduced into the body to protect against disease. If enough of the population is vaccinated, herd immunity provides protection to unvaccinated individuals.

A **vaccine** is a preparation of a harmless foreign antigen that is deliberately introduced into the body to protect against a specific disease. The antigen in the vaccine is usually some part of the pathogen. It triggers the immune system to produce antibodies against the antigen but it does not cause the disease. The immune system remembers its response and will produce the same antibodies if it encounters the antigen again. If enough of the population is vaccinated, herd immunity (indirect protection) provides unvaccinated individuals in the population with a measure of protection against the disease. There are two basic types of vaccine: subunit vaccines and whole-agent vaccines (below).





Whole-agent vaccine

Contains whole, non-virulent microorganisms

Inactivated (killed)

Viruses for vaccines may be inactivated with formalin or other chemicals. They present almost no risk of infection, e.g. most influenza vaccines, Salk polio vaccine.

Attenuated (weakened)

Attenuated viruses are usually strains in which mutations have accumulated during culture. These live viruses can back-mutate to a virulent form, e.g. MMR vaccine.

Subunit vaccine

Contains some part or product of microbes that can produce an immune response. Includes vaccines made using genetic engineering, inactivated toxins, and conjugated and **acellular vaccines**, e.g. the diphtheria-tetanus-pertussis vaccine and the vaccine against bacterial meningitis.

Why are vaccinations given?

CDC

Vaccines against common diseases are given at various stages during childhood according to an immunisation schedule. Vaccination has been behind the decline of some once-common childhood diseases, such as mumps and measles.

Most vaccinations are given in childhood, but adults may be vaccinated against a disease (e.g. TB, tetanus) if they are in a high risk group (e.g. the elderly or farmers) or to provide protection against seasonal diseases such as influenza.

Tourists may need specific vaccines if the country they are visiting has a high incidence of a certain disease. For example, travellers to South America should be immunised against yellow fever, a disease that does not occur in Australia.

1. (a) What is a vaccine? _____

_____

(b) Provide some examples of when vaccinations are needed: _____

_____

_____

_____

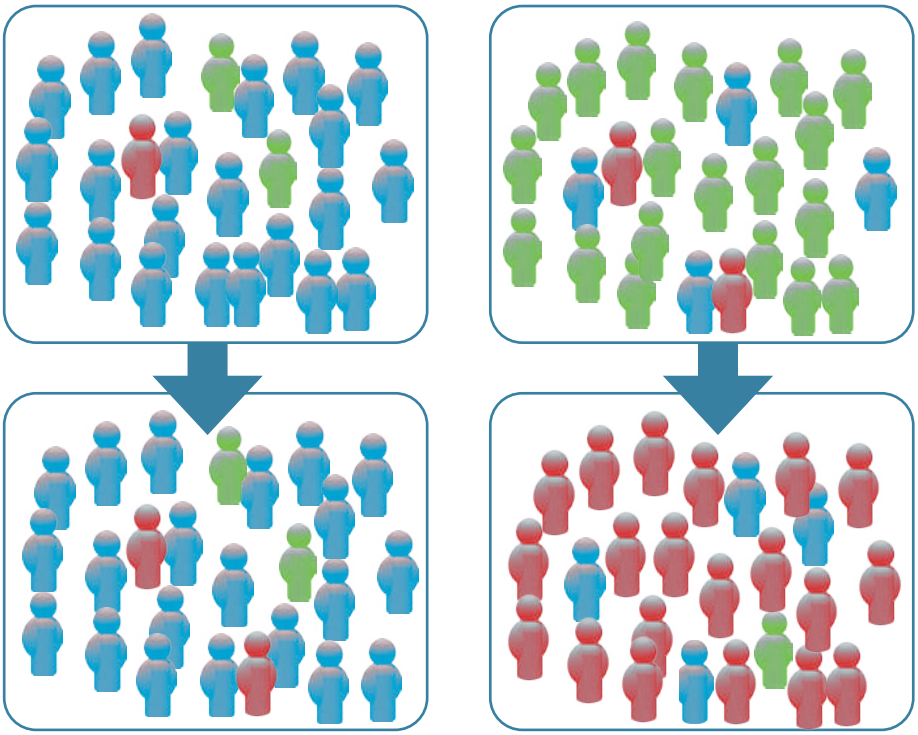
ISBN: 978-1-98-856655-9

Vaccination can provide herd immunity

- **Herd immunity** occurs when the vaccination of a significant portion of a population provides some protection for individuals who have not developed immunity (e.g. have not been vaccinated and are not immunised). In order to be effective for any particular disease, a high percentage of the population needs to be vaccinated against that disease. High vaccination rates make it difficult for the disease to spread because there are very few susceptible people in the population.
- Herd immunity is important for people who cannot be vaccinated (e.g. the very young, people with immune system disorders, or people who are very sick, such as cancer patients).

High herd immunity: Most of the population is immunised. The spread of the disease is limited. Only a few people are susceptible and become infected.

Low herd immunity: Only a small proportion of the population is immunised. The disease spreads more readily through the population, infecting many more people.



Immunised and healthy

Not immunised and healthy

Not immunised, sick and contagious

DID YOU KNOW?

The level of vaccination coverage to obtain herd immunity differs for each disease. Highly contagious diseases (e.g. measles) need a much higher vaccine uptake (95%) than a less contagious disease such as polio (80-85%).

2. Attenuated viruses provide long term immunity to their recipients and generally do not require booster shots. Why do you think attenuated viruses provide such effective long-term immunity when inactivated viruses do not?

3. (a) What is herd immunity?

(b) Why are health authorities concerned when the vaccination rates for an infectious disease fall?

4. Some members of the population are unable to be vaccinated. Give an example and explain why herd immunity is very important to them:

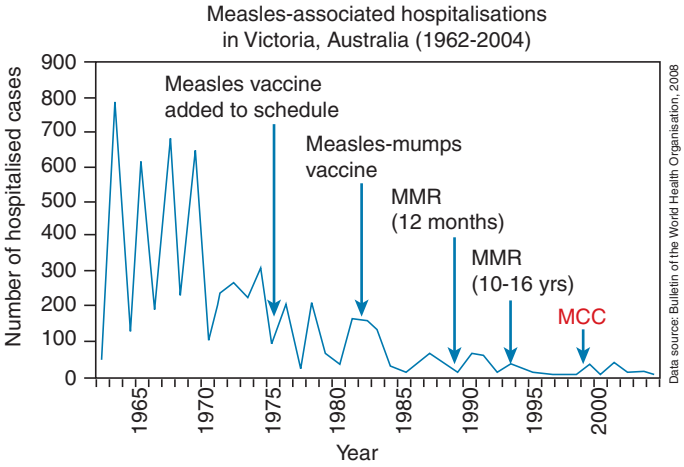
ISBN: 978-1-98-856655-9

The effect of vaccine on infectious disease

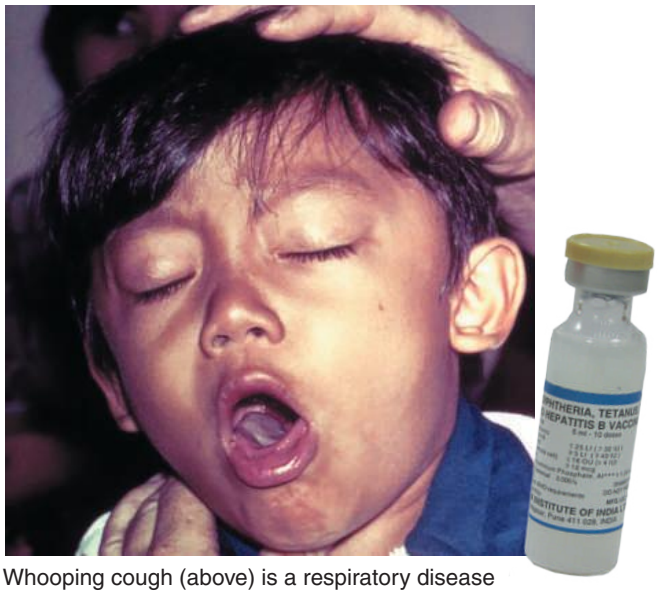
- To date, the only infectious disease globally eradicated has been smallpox. Several factors led to this success. Smallpox is easily identifiable by its characteristic rash, making surveillance and containment of infected patients easier. It has no other natural carriers so, once immunisation rates reached a critical level, its spread through the population was limited.
- Other diseases can be more difficult to eradicate. This is especially true for diseases that have a long period between infection and the symptoms showing (e.g. TB) or diseases caused by pathogens with high rates of mutation (e.g. *influenzavirus* or HIV).

Measles elimination in Australia

Measles is a highly contagious disease. One infected person can infect 12-18 people during their infectious period. In 2014, the World Health Organisation (WHO) announced that measles had been eliminated from Australia. High vaccination rates contributed to its elimination. However, measles still occurs in other countries so it could be reintroduced if an infected traveller entered Australia. Maintaining high levels of vaccination is important in preventing its reintroduction.



The graph above shows the role of vaccination in reducing measles hospitalisations in the state of Victoria.
MMR is the introduction of the measles/mumps/rubella vaccine.
MCC (measles control campaign) was an extensive mass vaccination and monitoring campaign.



CDC

Whooping cough (above) is a respiratory disease caused by the bacterium *Bordetella pertussis*. Despite high vaccination rates, whooping cough is increasing in Australia. Several factors may be contributing to this.

- Until 1997, a whole vaccine was used. It contained hundreds of different antigens and provided protection against many strains of the pertussis pathogen. In 1999, an acellular vaccine, which does not contain the whole pathogen, was introduced (inset above). It only contains five antigens and so provides less protection.
- New strains of *B. pertussis* are evolving, and the new vaccine is not effective against them.
- More adults who were vaccinated against whooping cough in childhood are contracting the disease. This suggests that the effectiveness of the vaccine declines over time.

5. The graph above provides long term immunity data for measles in Victoria. Use this data to provide evidence for the role of vaccination programmes in eliminating measles from Australia:

6. What could happen if vaccination rates for measles fell too low? _____

7. (a) Why could the change to a new vaccine have affected the rates of whooping cough in Australia?

   (b) Why do you think a new vaccine was introduced? _____

ISBN: 978-1-98-856655-9

182 Quarantine and Biosecurity

Key Idea: Quarantine is a way of ensuring that diseases do not enter a new area. It is an important part of biosecurity.

Biosecurity is important for Australia because its relative isolation means that it has managed to exclude many of the pathogens infecting plants and animals in other countries. Precautions such as **quarantine**, which isolates exposed individuals that may be infected, as well as screening international travellers and imported produce, help to limit the entry of diseases into Australia. Quarantine is distinct from **isolation**, which aims to contain disease by isolating an already infected person. Quarantine played an important role in reducing the number of cases of Covid-19 entering the Australian population and reduced the initial spread of the virus in the community.

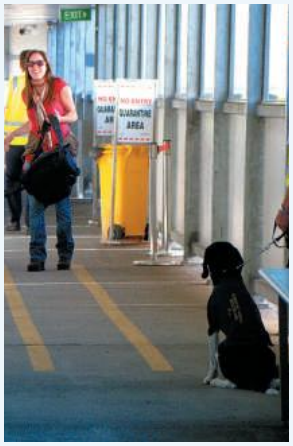
Biosecurity in Australia

Australia has strict biosecurity rules and measures in place to prevent the entry of pests and diseases into the country. For most people this is most visible in airports when entering Australia from overseas. Passengers disembarking from aircraft are repeatedly reminded of what can't be brought into the country and instant fines are given to those who ignore the warnings. Inspection officers use X-ray machines and detector dogs to check luggage for prohibited goods, especially fresh food or animal and plant materials.

Biosecurity inspections are also made on goods entering on cargo ships. Shipping containers are inspected for unwanted plants or animals that may have entered the container when loading. This is common in fresh food containers (e.g. fruits). If pests are found, the containers may be turned away. Inspection of many goods occurs at the home port before they are loaded on to cargo ships.

Goods PROHIBITED entry into Australia without authorisation

- Milk and dairy products
- Seeds and beans
- Popping corn and raw nuts
- Eggs and egg products
- Fresh fruit and vegetables
- Live animals
- Meat and fish products
- Live plants
- Biological materials (e.g. human/animal vaccines)
- Deer horn/velvet
- Soil and sand



Bjorn Christian Tørrissen CC3.0

The role of quarantine

When organisms are brought into Australia they must undergo a quarantine period to monitor health and ensure that no pests or diseases are in or on the organism. Quarantine may also apply to travellers who have been in contact with infected persons or have returned from places experiencing disease outbreaks. These procedures were used during the SARS epidemic in 2003, the swine flu pandemic in 2009, and the Covid-19 pandemic.

The equine industry is an important part of the Australian economy. Live horses entering the country are quarantined to check for diseases that may affect the industry. In August 2007, equine influenza was found in horses at the Eastern Creek Quarantine Station, the first time it had entered Australia. Somehow, it escaped quarantine and spread throughout NSW and into Queensland. As a result of strict non-movement orders, the outbreak was contained by February 2008.

Equine influenza is easily spread.

1. Why is biosecurity important for Australia? _____

2. Why is pre-inspecting goods at the home port a useful biosecurity measure? _____

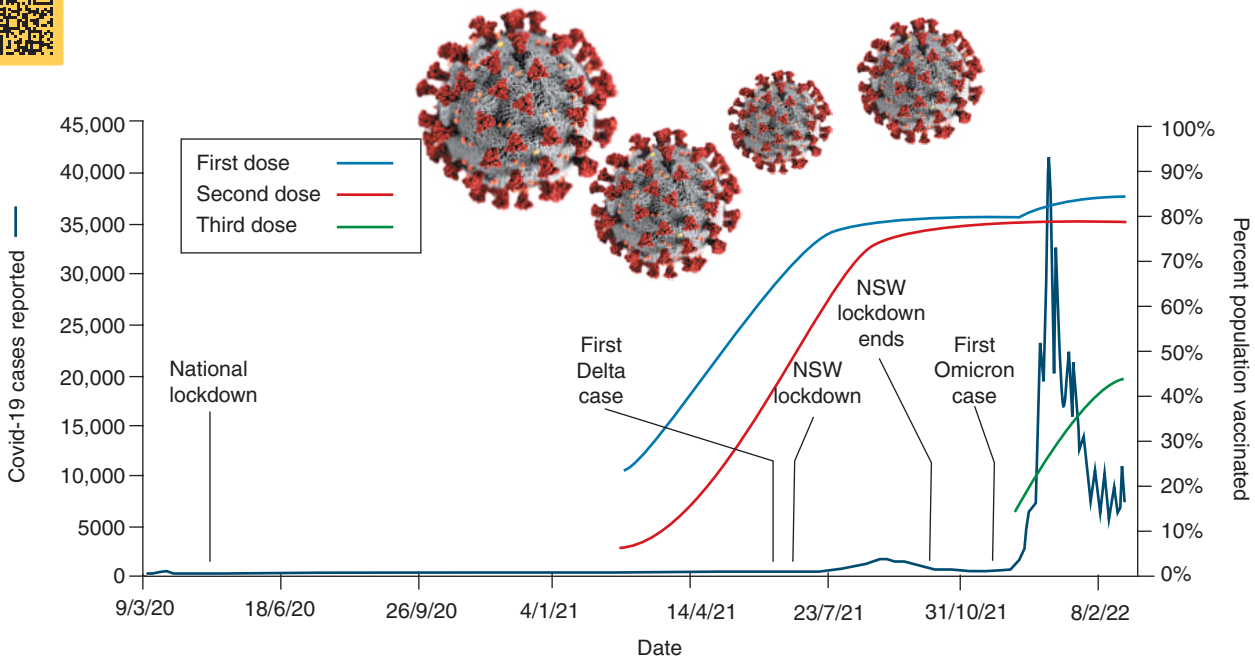
3. How does quarantine prevent the spread of disease? _____

4. How does Australia's geographic position help prevent the entry and spread of disease in Australia? _____

ISBN: 978-1-98-856655-9

The effectiveness of lock-downs in containing Covid-19

- Early in 2020 Australia implemented strict measures to keep infections of SARS-CoV-2 under control. These included closing the border to everyone except returning Australian citizens. Lockdown measures were implemented, where citizens were required to stay home and all but essential businesses were required to close.
- These measures became important when more infectious strains (delta and omicron) of the virus eventually entered the community. Even with these controls in place, the highly infectious omicron strain still infected tens of thousands of people.



5. Why did Covid-19 (delta) cases continue to rise even after NSW entered a strict lockdown? _____

6. During the Covid-19 delta outbreak there were state-wide restrictions on movement and gatherings in NSW. These restrictions were lifted near the end of delta's peak. Very few restrictions were in place for the Covid-19 omicron outbreak, even though it was a much more infectious strain. Describe the shape of the delta and omicron curves, and how state-wide restrictions and lockdowns might have influenced these:

7. During the NSW Covid-19 delta lockdown the goal of reaching 70% of people fully vaccinated was given before the lockdown could end. Why would setting a clear goal for a lockdown play an important part in reducing cases of Covid-19 (or any other disease) and maintaining morale?

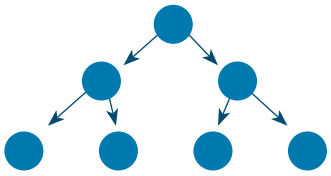
ISBN: 978-1-98-856655-9

183 Modelling Disease Outbreak and Spread

Key Idea: Being able to model the spread of a disease can help predict where, when, and how it will spread.
Modelling how a disease spreads can help preparation for an eventual outbreak. Elements of the model must account for how infectious a pathogen is and for how long, the density and mobility of the population, and even the level of mortality of infected people. These models can be used to test the effectiveness of public health measures.

Modelling a disease

- A spreadsheet can be used to model the spread of disease. There are also numerous online models that can be used.
- In the most simple model (right) whenever an infected person meets another, a new infection occurs. The number of interactions at each infection cycle affects the spread of the disease.
- Using a spreadsheet, you will first model an infected person meeting (and infecting) two other people. In this model, once the infected person has infected two people they are no longer infectious.



A simple infection model. One person infects two, who infect two more...

Investigation 12.2 Modelling disease outbreak and spread

See appendix for equipment list.

1. Working in pairs, enter the following into a spreadsheet:

	A	B
1	New infections	Total infections
2	1	=SUM($A$2:A2)
3	=A2*2	=SUM($A$2:A3)
4	=A3*2	=SUM($A$2:A4)
5	=A4*2	=SUM($A$2:A5)
6	=A5*2	=SUM($A$2:A6)
7	=A6*2	=SUM($A$2:A7)
8	=A7*2	=SUM($A$2:A8)
9	=A8*2	=SUM($A$2:A9)

One infection cycle. Copy this down to row 12 (10 cycles of interactions).

1. How many new infections are there per infection cycle after 10 infection cycles? _____
2. How many infected people are there in total after 10 infection cycles? _____

2. Now set the interactions per infected person to 3 (A2*3) and reset the model.

3. How many new infections are there per cycle of infection after 10 infection cycles? _____
4. How many infected people are there after 10 cycles of infection? _____

3. We can now extend the model by adding in a little randomness. The number of people interacting with each infected person may not always be the same. In our extended model, we shall randomise the number of people interacting to between 1 and 4.

	A	B	C
1	New infections	People interacted with per person	Total infected people
2	1	=RANDBETWEEN(1,4)	=SUM($A$2:A2)
3	=A2*B2	=RANDBETWEEN(1,4)	=SUM($A$2:A3)
4	=A3*B3	=RANDBETWEEN(1,4)	=SUM($A$2:A4)
5	=A4*B4	=RANDBETWEEN(1,4)	=SUM($A$2:A5)
6	=A5*B5	=RANDBETWEEN(1,4)	=SUM($A$2:A6)
7	=A6*B6	=RANDBETWEEN(1,4)	=SUM($A$2:A7)
8	=A7*B7	=RANDBETWEEN(1,4)	=SUM($A$2:A8)
9	=A8*B8	=RANDBETWEEN(1,4)	=SUM($A$2:A9)
10	=A9*B9	=RANDBETWEEN(1,4)	=SUM($A$2:A10)

Add new infections to total from previous row (cycle)

Generates a random number between 1 and 4

Calculates the total number of people infected

5. Run the model five times by recalculating the spreadsheet using the **recalculate** or **calculate now** option (depending on your spreadsheet). On average, how many people in total have been infected after ten cycles?

_____

_____

ISBN: 978-1-98-856655-9

4. Not all interactions will result in an infection. The pathogen may not be highly infectious or the correct mode of transmission may not have occurred (for example, a person with a cold may have been careful where and how they coughed).

5. First we need to decide the probability of each interacting person being infected. For this model, we will say there is a 50% chance that any interacting person will be infected. We shall first produce a random number between 0 and 1 (see * below). We can now use this block of infected (1) or not infected (0) cells in our model. Once the formula is set up, you can recalculate the spreadsheet to obtain different infection scenarios.

	A	B	C	D
1	New infections	People interacted with per person	Infected people	Total infected people
2	1	=RANDBETWEEN(1,4)	=IF(B2=1,$B$15,IF(B2=2,$B$15+$B$16,IF(B2=3,$B$15+$B$16+$B$17,IF(B2=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A2)
3	=A2*C2	=RANDBETWEEN(1,4)	=IF(B3=1,$B$15,IF(B3=2,$B$15+$B$16,IF(B3=3,$B$15+$B$16+$B$17,IF(B3=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A3)
4	=A3*C3	=RANDBETWEEN(1,4)	=IF(B4=1,$B$15,IF(B4=2,$B$15+$B$16,IF(B4=3,$B$15+$B$16+$B$17,IF(B4=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A4)
5	=A4*C4	=RANDBETWEEN(1,4)	=IF(B5=1,$B$15,IF(B5=2,$B$15+$B$16,IF(B5=3,$B$15+$B$16+$B$17,IF(B5=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A5)
6	=A5*C5	=RANDBETWEEN(1,4)	=IF(B6=1,$B$15,IF(B6=2,$B$15+$B$16,IF(B6=3,$B$15+$B$16+$B$17,IF(B6=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A6)
7	=A6*C6	=RANDBETWEEN(1,4)	=IF(B7=1,$B$15,IF(B7=2,$B$15+$B$16,IF(B7=3,$B$15+$B$16+$B$17,IF(B7=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A7)
8	=A7*C7	=RANDBETWEEN(1,4)	=IF(B8=1,$B$15,IF(B8=2,$B$15+$B$16,IF(B8=3,$B$15+$B$16+$B$17,IF(B8=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A8)
9	=A8*C8	=RANDBETWEEN(1,4)	=IF(B9=1,$B$15,IF(B9=2,$B$15+$B$16,IF(B9=3,$B$15+$B$16+$B$17,IF(B9=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A9)
10	=A9*C9	=RANDBETWEEN(1,4)	=IF(B10=1,$B$15,IF(B10=2,$B$15+$B$16,IF(B10=3,$B$15+$B$16+$B$17,IF(B10=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A10)
11	=A10*C10	=RANDBETWEEN(1,4)	=IF(B11=1,$B$15,IF(B11=2,$B$15+$B$16,IF(B11=3,$B$15+$B$16+$B$17,IF(B11=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A11)
12	=A11*C11	=RANDBETWEEN(1,4)	=IF(B12=1,$B$15,IF(B12=2,$B$15+$B$16,IF(B12=3,$B$15+$B$16+$B$17,IF(B12=4,$B$15+$B$16+$B$17+$B$18))))	=SUM($A$2:A12)
13				
14				
15	=RAND()	=IF(A15>0.5,1,0)		
16	=RAND()	=IF(A16>0.5,1,0)		
17	=RAND()	=IF(A17>0.5,1,0)		
18	=RAND()	=IF(A18>0.5,1,0)		
19				
20				
21				

* Produces a 50% probability of infection

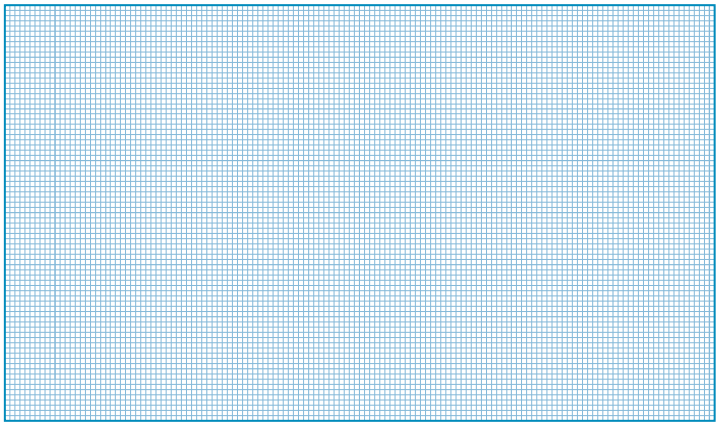
The "IF" statement incorporates the number of interactions and the probability of infection into the model

6. Run the model five times by recalculating the spreadsheet as before. On average, how many people in total have been infected after ten cycles now?

7. The third model above is much more realistic than the first, but still lacks many factors that would affect the model outcome. List at least three factors that could be added to the model to make it even more realistic:

Modelling with S, I, and R

- A more advanced predictive mathematical model than your spreadsheet called SIR can be used to show the transmission of infectious diseases. In this model there are three compartments: **S** (the number of susceptible individuals), **I** (the number of infected individuals), and **R** the number removed (those who have been removed through recovery or death).
- The data in the table (below right) is a theoretical example. It assumes a closed system (e.g. a single state with no travel), no prior immunity (everyone is susceptible), no vaccine, and no physical distancing or other precautionary measures in place.



Week	S	I	R
0	7,000,000	2	0
1	6,999,986	15	1
2	6,999,881	113	8
3	6,999,090	847	65
4	6,993,162	6352	488
5	6,948,741	47,597	3664
6	6,618,002	354,538	27,462
7	4,271,669	2,523,602	204,731
8	0	5,533,470	1,466,532
9	0	2,766,735	4,233,267
10	0	1,383,368	5,616,634
11	0	691,684	6,308,318
12	0	345,842	6,654,160
13	0	172,921	6,827,081
14	0	86,460	6,913,542

8. Plot the tabulated SIR data (right) on the grid provided. Plot all three data sets on one axis and add a key.

ISBN: 978-1-98-856655-9

9. Describe the relationship between the three compartments (S,I,and R) over time: _____

Mathematical models and vaccination

Mathematical models of the effect of vaccination on populations have been used since the mids 1700s. In 1760, Swiss mathematician Daniel Bernoulli published a study on the effect of immunisation with cowpox (against smallpox) on the life expectancy of the immunised population. Around the time of the First World War, Ronald Ross produced mathematical models to show that malaria could be controlled without removing every last mosquito. These mathematical models are the basis for many vaccination programmes and show why herd immunity is an important aim of public health programmes.

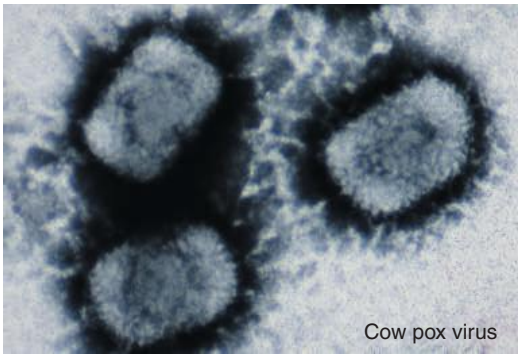
- For the incidence of disease to decline, every case or primary infection should generate less than one other case or secondary infection (on average).
- The number of secondary infections caused by an infectious individual is denoted as R. $\mathbf{R_0}$ (R nought) is the basic reproductive number of the pathogen. It is the number of secondary infections caused by a primary infection introduced into a wholly susceptible population.
- $\mathbf{R_{0p}}$ is the basic reproductive number under vaccination. It is the number of secondary infections caused by a primary infection introduced into a population where a proportion (*p*) of the population is vaccinated. For a perfect vaccination that confers life long immunity:

$$\mathbf{R_{0p} = (1 - p)\, R_0}$$

- p_c is the critical vaccination proportion that will achieve eradication. To achieve this proportion, the basic reproductive number under vaccination (R_{0p}) must be just less than 1, so:

$$\mathbf{p_c = 1 - \frac{1}{R_0}}$$

Calculating p_c requires estimates of R_0 (right)



Cow pox virus

Estimates of R_0 for populations and dates

Infection	Location	Date	R_0
Measles	Senegal	1964	18
Smallpox	West Africa	1960	2.3
Mumps	UK	1987	8
Rubella	USA	1967	6
Influenza	UK	2010	1.5
Covid-19 (Δ)	Global	2021	~6.5

10. How are mathematical and computer models useful in controlling disease? _____

11. What is the critical vaccination proportion for each of the following diseases?

    (a) Measles: _____

    (b) Influenza: _____

    (c) Covid-19: _____

12. Why is the p_c of measles so high? _____

13. Calculating the R_0 of Covid-19 was a critical step in controlling the spread of the virus. What does the R_0 of Covid-19 tell us? Could the disease have been controlled without a vaccine, or is vaccination the only way to stop its spread?

ISBN: 978-1-98-856655-9

184 Controlling Viral Disease

Key Idea: Breaking the chain of infection from person to person prevents the spread of viruses in a population.

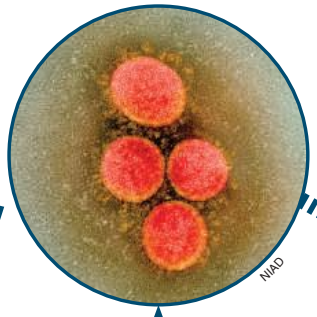
Many factors can influence the spread of a viral disease in a population including the social climate, and people's diet, general health, and access to medical care. Vaccination, and modification of people's behaviour, such as improving hygiene practices, can reduce the transmission rate of some diseases and inhibit their spread. One of the most important ways of controlling the spread of a virus or other pathogen is by breaking the chain of infection. Parts of this chain can be targeted to prevent new viruses from entering the community or to prevent their spread.

Containing a viral disease: Covid-19

Breaking the chain of infection

Host susceptibility is an important link in the chain. Covid-19 spreads quickly in areas where people live close together and infectious people do not take steps to keep themselves away from others. Cultural differences between patients and medical workers, different countries, and, in some cases, mistrust of the authorities increase the risk of spread.

Causative agent



The exact reservoir (primary host) of Covid-19 is still not known, although it is likely to be the horseshoe bat family. Whether or not the virus is widespread or a novel strain is also not known.

Susceptible host

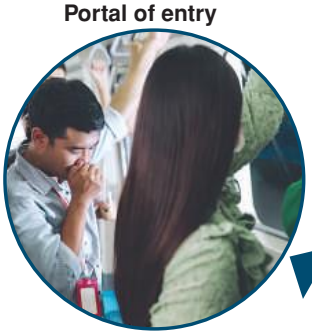
Reservoir

The causative agent is the pathogen responsible for the infection, e.g. the SARS-CoV-2 virus. Eliminating this part of the chain from the environment is often the most difficult. It is likely the SARS-CoV-2 virus will never be fully eliminated.

Covid-19 chain of infection

Targeting the transmission of the virus between people is one of the most effective ways to prevent infection. Preventing Covid-19 infection is based on good hygiene practices, which include washing hands, disinfecting high touch surfaces (e.g. door handles), and wearing masks in public places.

Portal of entry



Portal of exit

Mode of transmission

The portal of entry is how the organism enters a host. Covid-19 can enter the body through the nose and mouth.

The portal of exit describes how the organism leaves the reservoir or host. It is still not known exactly how Covid-19 crossed from animals to humans, but it was possibly from sick animals in crowded markets.

1. Describe two important parts in the Covid-19 chain of infection that could result in continual infection of human populations:

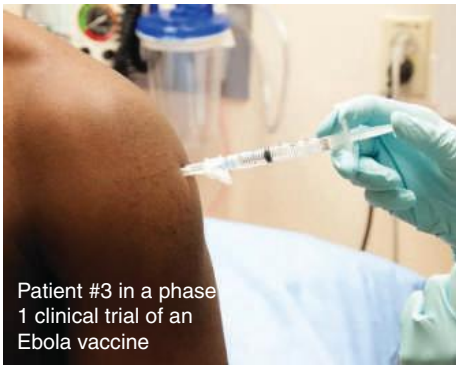
ISBN: 978-1-98-856655-9

2. Which part/parts of the Covid-19 chain of infection is/are likely the best to focus on in the current pandemic in order to break the chain of infection? Explain your answer.

Case study: Controlling Ebola in West Africa

- Recall that between 2014 and 2016 there was a widespread outbreak of Ebola virus disease (EVD) in West Africa. At the start of this outbreak, there was very little in the way of drug therapy that could be used to treat the disease, despite there having been previous outbreaks.
- Vaccines for EVD were rare and mostly experimental, and antiviral drugs were even more so. The 2014 outbreak was so widespread and rapid that traditional methods of isolating infected people and tracking down all contacts were not effective. The infection of medical workers prompted the need for drugs to treat patients. However, few were available and none had been tested on humans. Questions were raised: was it ethical to give infected patients drugs that had not been through rigorous testing and had not been tested on humans?
- Trials of untested drugs for the Ebola epidemic were approved by WHO on August 11, 2014. There were many qualifiers added to the approval, including freedom of choice for those treated, and that information gained must be freely shared. Human trials of a livestock-based vaccine began in Liberia and Guinea early in 2015 but, because the number of EVD cases is declining, it will be difficult to determine if the vaccine was effective.

Treatment centre in Guinea


Patient #3 in a phase 1 clinical trial of an Ebola vaccine

CDC

Points on ethics

- There is limited drug supply – who should be treated first? This includes questions around should it be the youngest, the sickest, or the most likely to benefit?
- Who should pay for the production of the drug? The countries involved in the outbreak are some of the poorest in Africa.
- New drugs would be used primarily in Africa, but are made outside Africa by non-African companies. Is it acceptable to treat Ebola victims in Africa as test subjects?

3. Hold a class discussion on the ethics of using untested drugs during an epidemic. Write a brief summary of the points raised here and include your own opinion on the matter:

4. Since the introduction of the Covid-19 vaccine there have been, throughout the world, many protests against what some see as governments forcing mass vaccination programmes on their people via vaccination mandates, lockdowns, etc. Many would argue that, during such unprecedented times, the greater good of the population is more important that an individual's right to choose whether or not to be vaccinated. Many would argue the opposite.

   What is your opinion on the matter? You may wish to hold a class debate or discussion. What if Covid-19 was far more deadly or infectious, or a zombie virus, as so often depicted in movies, began to spread? Would this change your opinion?

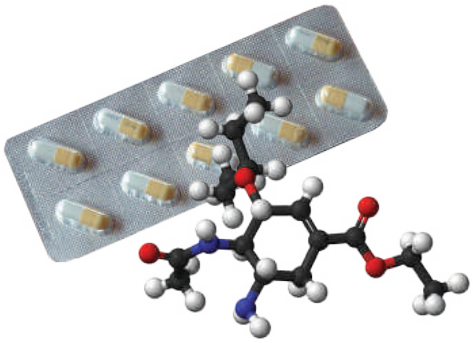
ISBN: 978-1-98-856655-9

185 Antiviral Drugs

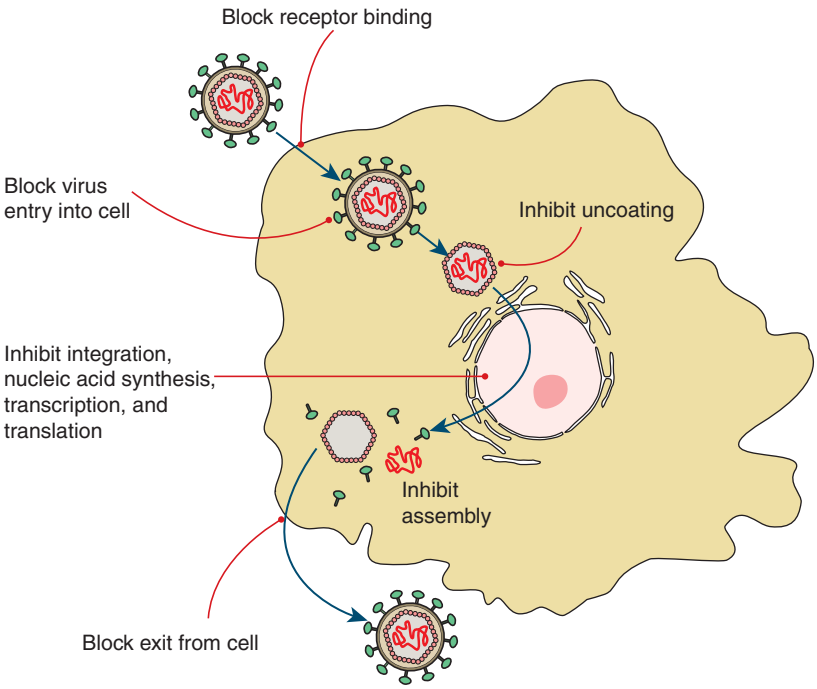
Key Idea: Antiviral drugs target key points in a viral life cycle. Because viruses do not display the characteristics of living things, antiviral drugs do not focus on destroying the virus itself, but on preventing completion of the viral life cycle. This is done by blocking the entry of the virus into cells or by inhibiting key steps in viral replication (below).

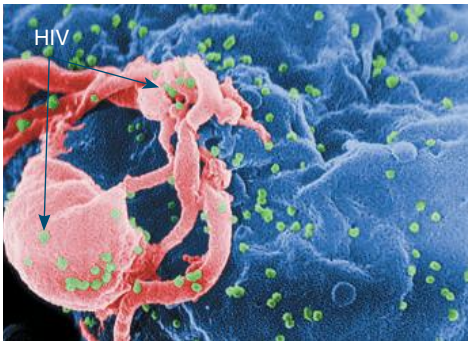
Actions of antiviral drugs

Currently, antiviral drugs are available for use against HIV, hepatitis, influenza, and the herpes virus. Most antiviral drugs work by inhibiting replication of the virus. The drug stops the virus replicating, giving the immune system time to destroy the virus. Antiviral drugs work on several parts of virus life cycle (right).

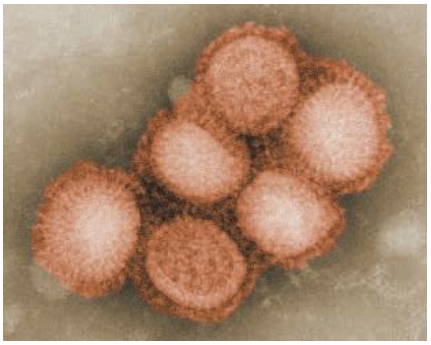


Oseltamivir (marketed as Tamiflu, above) slows the spread of influenza A and B viruses in the body by preventing a newly formed virus particle from budding from an infected cell. However, various trials have shed doubt on whether the drug produces any real benefit to flu sufferers.

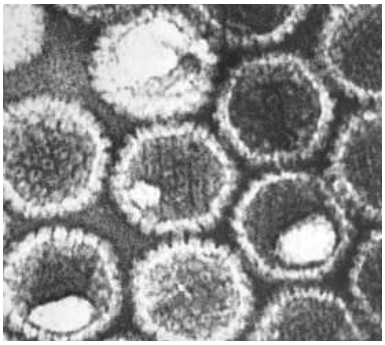




HIV is treated using Highly Active Antiretroviral Therapy (HAART). HAART usually consists of two different nucleoside analogue reverse transcriptase inhibitors (NARTIs) and a protease inhibitor. NARTIs block the reverse transcriptase enzyme used to generate DNA from viral RNA, thereby blocking the integration of viral genes into the T cell genome. Protease inhibitors block the HIV protease enzyme, which is essential to forming infectious virions.



Influenza is caused by either the influenza A or influenza B virus. Influenza A is treated using the drugs amantadine and rimantadine which block the uncoating of the virus inside the host cell. Influenza B is treated using the drugs zanamivir and oseltamivir (which are also active against influenza A). These two drugs inhibit the actions of neuraminidase, a glycoprotein on the viral surface, and stops the virus from exiting the cell.



Herpesvirus (a group of viruses that includes the viruses that cause cold sores and chickenpox) is treated with drugs that interfere with DNA replication by blocking the viral polymerase enzyme. Some drugs are activated by viral enzymes and are, therefore, only active in infected cells, providing a targeted treatment. Others are active in all cells and are less specific.

1. For each of the following mechanisms used by antiviral drugs, explain how it prevents the spread of the virus:

   (a) Blocking receptor binding: _____

   _____

   (b) Inhibiting assembly: _____

   _____

2. Why is using a combination of antiviral drugs with different actions more effective than using a single drug?

   _____

   _____

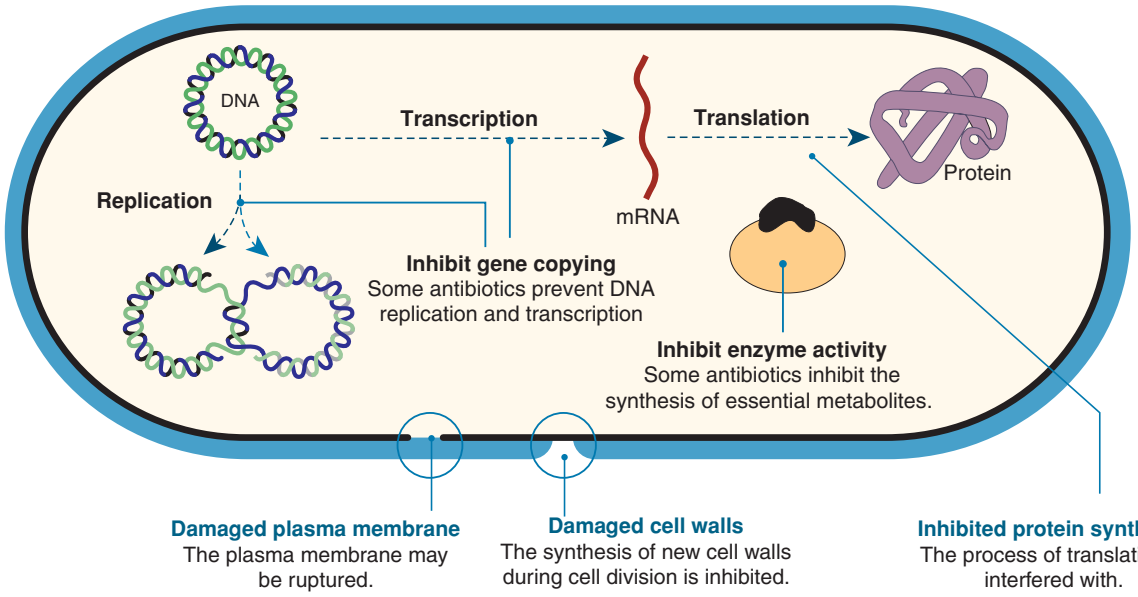
ISBN: 978-1-98-856655-9

186 Testing Antibiotics

Key Idea: Antibiotics are antimicrobial chemicals that kill bacteria (bactericidal) or inhibit their growth (bacteriostatic).

Antibiotics are chemicals that act against bacterial infections by either killing the bacteria (**bactericidal** action) or preventing them from growing (**bacteriostatic** action). Antibiotics interfere with bacterial growth by disrupting key aspects of bacterial metabolism (below). Antibiotics are ineffective against viruses because viruses lack the structure and metabolic machinery that antibiotics target. Antibiotics are produced naturally by bacteria and fungi to kill or inhibit competitors or pathogens, but most modern antibiotics are semi-synthetic modifications of these natural compounds.

How antibiotics work



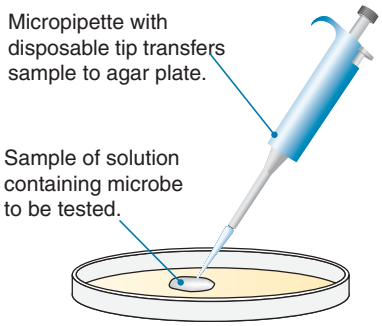
Damaged plasma membrane
The plasma membrane may be ruptured.

Damaged cell walls
The synthesis of new cell walls during cell division is inhibited.

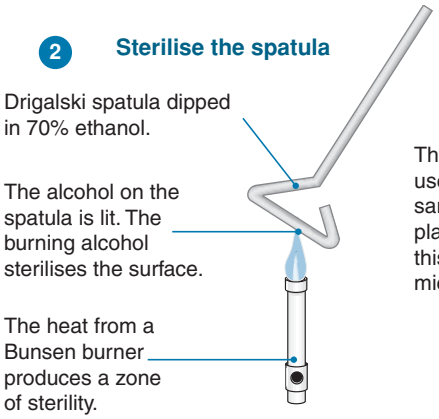
Inhibited protein synthesis
The process of translation is interfered with.

Testing effectiveness of antibiotics

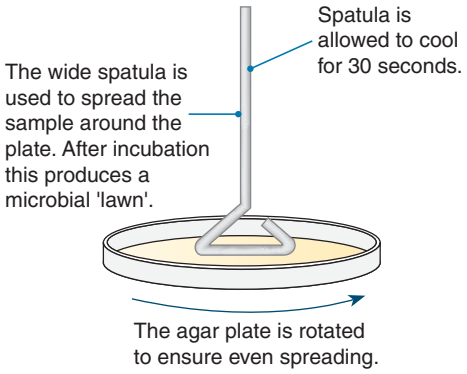
1 Pipette a sample



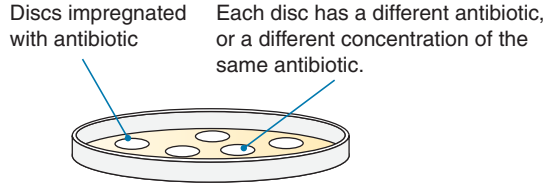
2 Sterilise the spatula



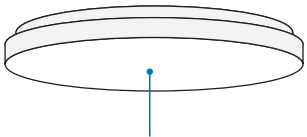
3 Spread the sample



4 Antibiotic discs

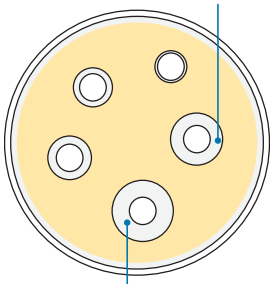


5 Incubate plate

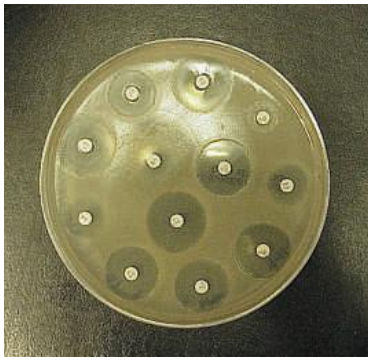


The plate is sealed and incubated upside down, preventing condensation dripping on the agar.

6 Analyse results



The size of the clear patch is measured and indicates the susceptibility of the bacteria to the antibiotic.



Clear area around antibiotic discs

ISBN: 978-1-98-856655-9

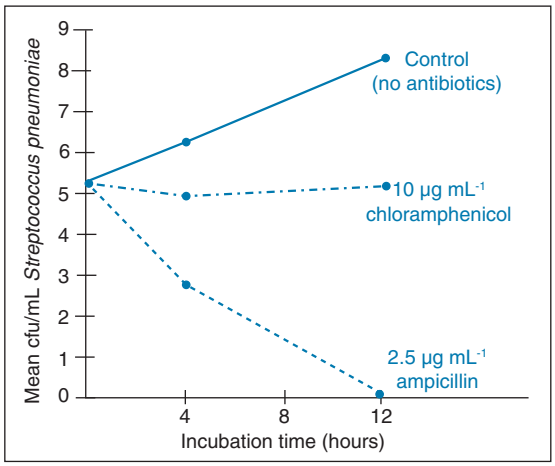
1. Why are viruses not affected by antibiotics? _____

2. Distinguish between bacteriostatic and bactericidal: _____

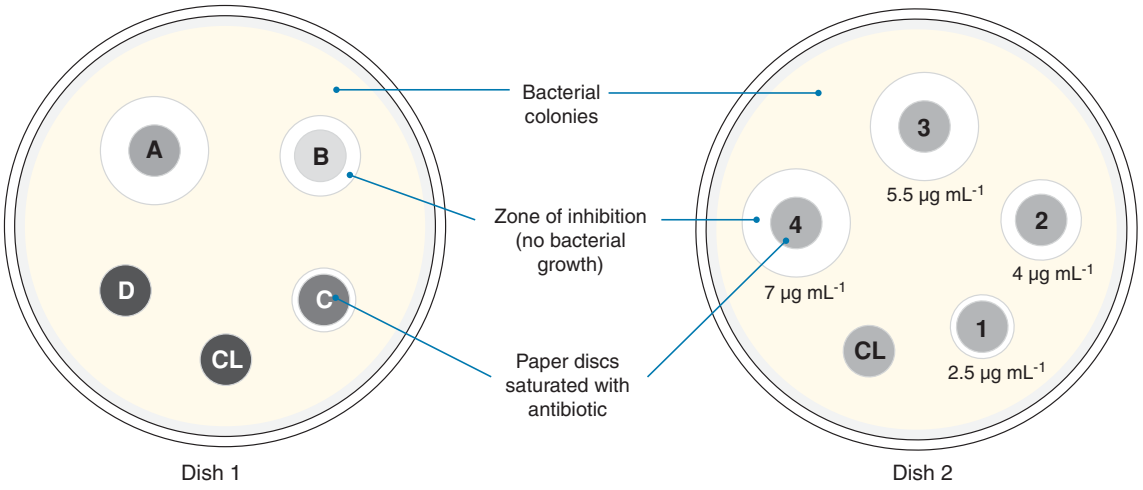
3. The graph (right) shows the effects of two antibiotics. Identify the antibiotic with a bacteriostatic action and the antibiotic with a bactericidal action. Explain your choice:

   Bacteriostatic: _____

   Bactericidal: _____



4. Two students carried out an experiment to determine the effect of antibiotics on bacteria. They placed discs saturated with antibiotic on petri dishes evenly coated with bacterial colonies. Dish 1 contained four different antibiotics labelled A to D and a control labelled CL. Dish 2 contained four different concentrations of a single antibiotic and a control labelled CL.



   (a) Which was the most effective antibiotic on Dish 1? _____

   (b) Which was the most effective concentration on Dish 2? _____

   (c) Explain your choice in question 5(b): _____

5. Referring to the procedure for testing antibiotics on the opposite page:

   (a) Why is the agar plate incubated upside down? _____

   (b) Why is the spatula dipped in alcohol and heated? _____

   (c) How would you measure the clear zone around the antibiotic discs? _____

ISBN: 978-1-98-856655-9

187 Incidence and Prevalence of Infectious Disease

Key Idea: The prevalence and incidence of infectious disease can help shape the responses of relevant health authorities.

Incidence and prevalence are important in the analysis of both infectious and non-infectious diseases in a population. **Prevalence** refers to the proportion of a population with a disease at a certain time, whereas **incidence** is the rate at which people develop the disease in a population. Understanding the prevalence and incidence of an infectious disease is important in developing health responses to the disease, including planning for future resource allocation such as intensive care units, or the quantity of antibiotics to manufacture or order.

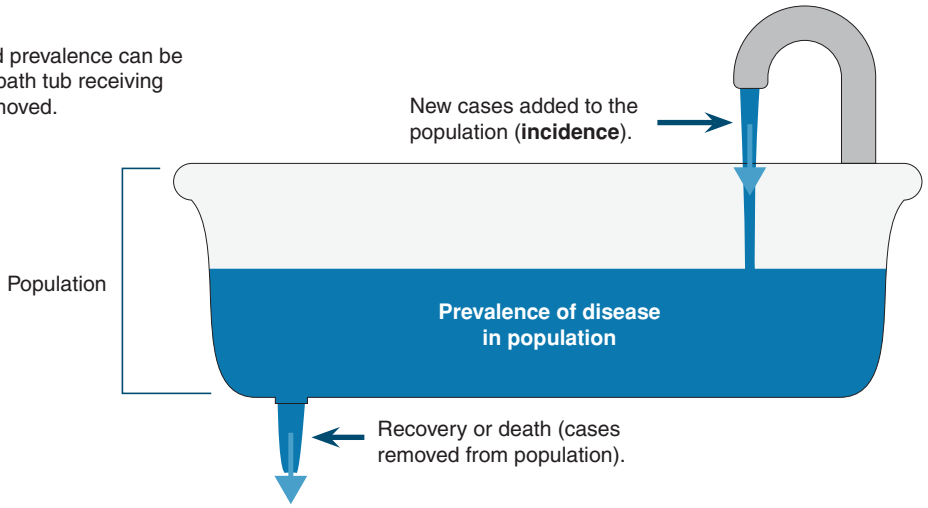
- Because different countries have different population sizes, comparing the number of cases of disease between countries requires the number of cases to be expressed per unit of population. The unit of population may be scaled to accommodate the size of the population or the scale of an outbreak, but is commonly per 1000 or 100,000 people.

$$\text{Prevalence of disease} = \frac{\text{All cases of disease during time period}}{\text{Population during time period}} \times \text{population unit (e.g. 100,000)}$$

$$\text{Incidence of disease} = \frac{\text{New cases of disease during time period}}{\text{Population during time period}} \times \text{population unit (e.g. 100,000)}$$

Incidence vs prevalence

The difference between incidence and prevalence can be demonstrated using the analogy of a bath tub receiving water from a tap, but with the plug removed.



Incidence and prevalence of dengue fever in Singapore

- Dengue fever is a mosquito-borne tropical disease caused by the dengue virus. Dengue fever lasts about two weeks from infection to recovery. The table below shows the new cases of dengue fever recorded in Singapore per week from the 23rd of January to the 5th of March 2022. The population of Singapore is 5,686,000.

Reporting week	23/1 - 29/1 2022	30/1 - 5/2 2022	6/2 - 12/2 2022	13/2 - 19/2 2022	20/2 - 26/2 2022	27/2 - 5/3 2022
New cases	171	154	169	206	199	264
Incidence (per 100,000)						
Total cases	171	325	323			
Prevalence (per 100,000)						

Data.gov.sg

1. Explain the difference between incidence and prevalence of a disease: _____

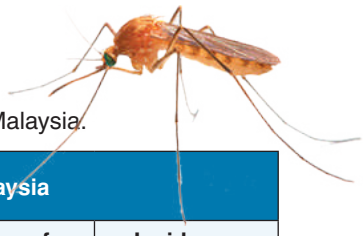
_____

_____

_____

2. Complete the table above, showing the incidence and prevalence of dengue fever in Singapore:

ISBN: 978-1-98-856655-9



Comparing dengue fever between countries

3. The data below shows the number of new cases of dengue fever per year, for Singapore and Malaysia.

Singapore				Malaysia		
Year	Population	Cases of dengue fever	Incidence (Per 100,000)	Population	Cases of dengue fever	Incidence (Per 100,000)
2011	5,184,000	5330		28,650,000	19,884	
2012	5,312,000	4632		29,070,000	21,900	
2013	5,399,000	22,170		29,470,000	43,346	
2014	5,470,000	18,326		29,870,000	108,698	
2015	5,535,000	11,294		30,270,000	118,961	
2016	5,607,000	13,085		30,680,000	97,593	
2017	5,612,000	2767		31,100,000	98,991	
2018	5,639,000	3285		31,530,000	76,964	
2019	5,704,000	15,998		31,950,000	127,065	

Data.gov.sg

(a) Complete the table by calculating the incidence of dengue fever in Singapore and Malaysia per year:

(b) In which years did an outbreak of dengue fever occur? _____

_____

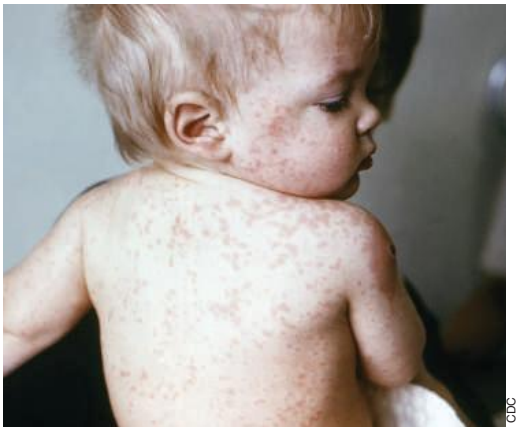
(c) Which country had the greater outbreak of dengue fever? _____

Vaccination and prevalence of disease

- Measles is a highly contagious disease for which an effective vaccination has been available for decades. However, that rate of vaccination differs between countries as a result of various political, social, and economic reasons. The table below shows the prevalence of measles, and vaccination rates for a selection of countries in 2015.

Country	Prevalence (cases per million)	Vaccination (% of 1 year olds vaccinated)
Afghanistan	34.21	63%
Argentina	0	89%
Australia	3.11	95%
Bahamas	0	94%
Ethiopia	177.68	56%
France	2.44	91%
New Zealand	2.17	93%
Somalia	539.04	46%
United States	0.59	92%

Our World in Data



CDC

4. (a) What evidence is there that vaccinating for measles reduces the prevalence of measles in a population?

_____

_____

_____

(b) What factors, other than vaccination, may have led to the low prevalence of measles in some of the countries above?

_____

_____

_____

ISBN: 978-1-98-856655-9

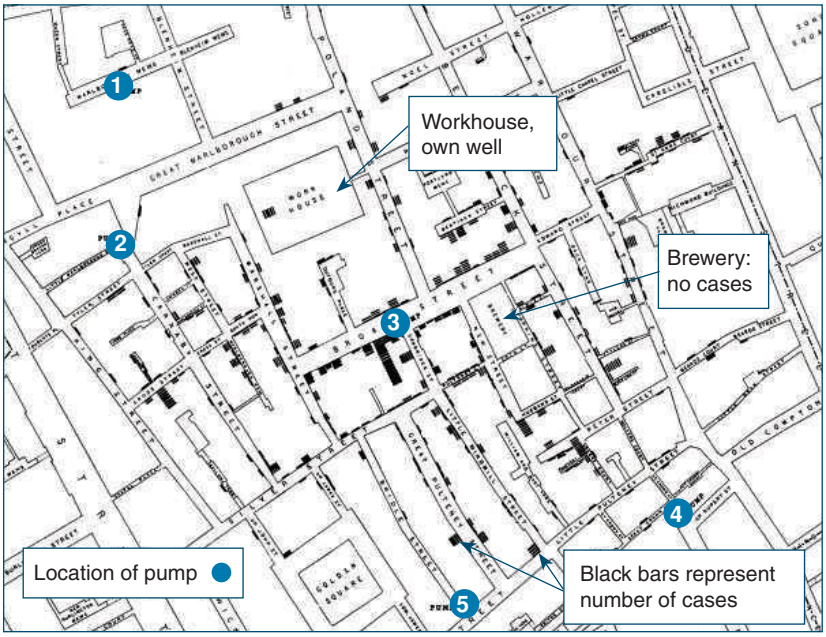
188 Evaluating Strategies for Controlling Disease

Key Idea: Understanding the nature of infectious disease has shaped how infectious diseases are controlled.

Infectious disease has always been a part of human civilisation. It wasn't until people began to understand that disease could be transferred by microscopic infectious pathogens that infectious diseases began to be truly controlled. However, even now, with all the medical knowledge humans have developed, we still see pandemics sweep across the globe every decade or so. Controlling a disease requires everyone affected to follow rules of hygiene and isolation. If those rules aren't followed the disease spreads. In most cases this means that diseases are controlled at a level where society operates without a disease causing any escalating effect, but the disease never goes away (e.g. flu). When those controls are not enough to suppress the disease, an epidemic, or pandemic can occur (e.g. Covid-19).

John Snow, The first epidemiologist

- In 1854, the area around Broad Street in London experienced an outbreak of cholera.
- The physician John Snow investigated the outbreak and he is often credited with ending it. Snow produced the map shown on the right. The black bars represent the number of cases of cholera in the area. The blue dots indicate the placement of water wells with pumps from which the population obtained water.
- He also studied the statistics of cholera deaths. Table 1 shows the number of cholera deaths for the areas of London and compares the number of cases to the elevation above the Thames (which was highly polluted at the time). Higher elevations sourced water upstream from London.
- By studying the data, Snow was able to locate the source of the cholera outbreak and convince authorities to take action to reduce its effects.



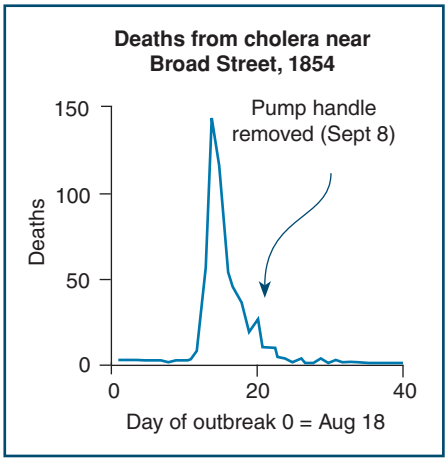


Table 1

Location in London	Elevation above Trinity high water mark (feet)	Population (1851)	Deaths from cholera	
			In five weeks ending Aug 12	In week ending Aug 19
West Districts	28	376,427	102	184
North Districts	135	490,396	62	38
Central Districts	49	393,256	58	32
East Districts	26	485, 522	168	105
South Districts	6	616,635	817	370
Total		2,362,236	1207	729

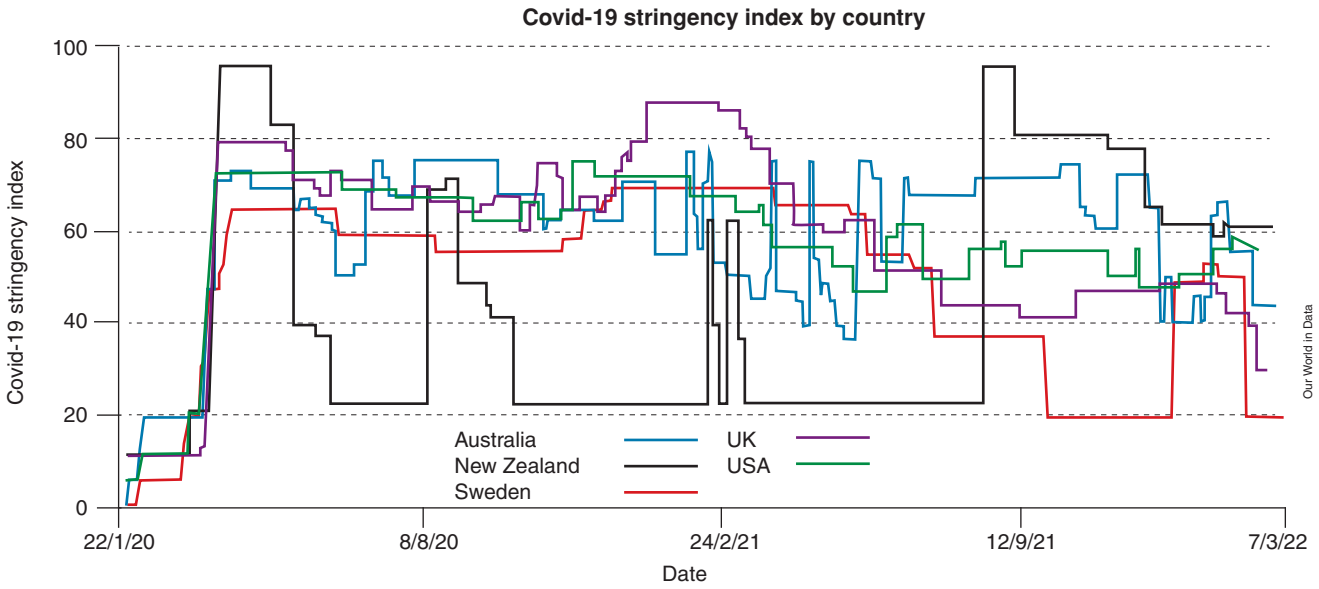
1. Use the data provided to locate the source of the cholera outbreak and write a statement arguing why it is the source, and what should be done to prevent further cases of cholera occurring:

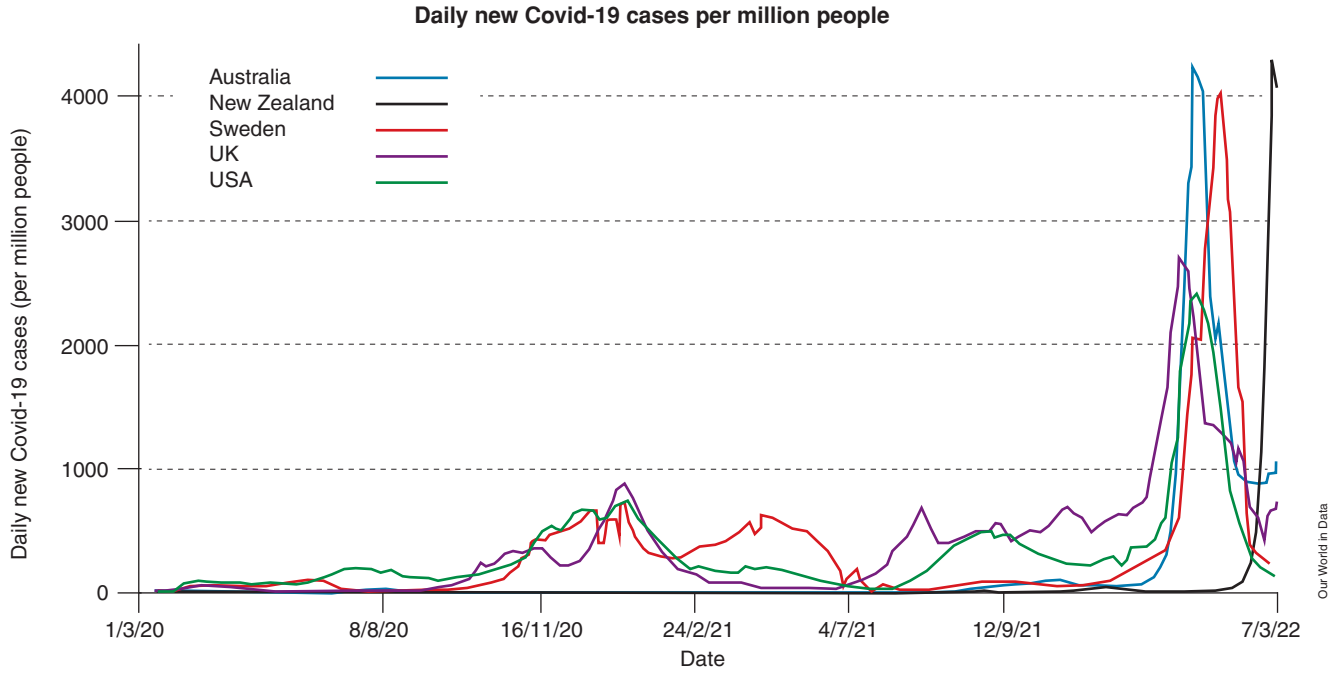
2. The pump handle from the offending well was removed (see graph above). Did this make any real difference in ending the outbreak?

ISBN: 978-1-98-856655-9

Responding to Covid-19

- How a country responds to a disease outbreak varies from outbreak to outbreak. It often depends on the type of disease, including how infectious it is and its mortality rate. It may also depend on a country's ideals and whether or not the new outbreak is perceived by the populace as a threat.
- The graphs below compare the stringency of a country's Covid-19 response to the number of new cases per day (incidence) of Covid-19. The stringency index is a combination of measures such as movement orders, mask wearing, school closures, etc.



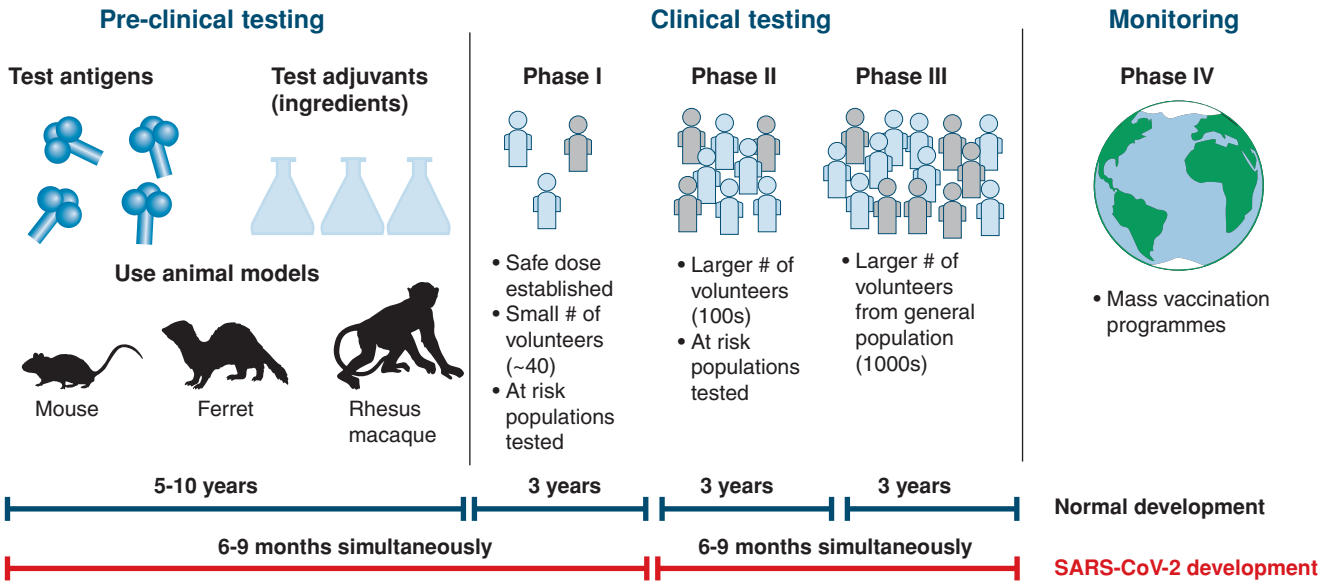


3. Is there a relationship between the stringency of a country's Covid-19 response and the number of cases per million in the country? Why or why not? You may need to do some extra research for a more complete answer. What other factors might be taken into account to understand the response of a country and the Covid-19 cases it experienced?

ISBN: 978-1-98-856655-9

Developing a Covid-19 vaccine

- The development of a Covid vaccine became a global priority when the seriousness of the virus was recognised. In order to produce the vaccine fast enough for it to be useful, vaccine development was fast tracked.
- A number of Covid vaccines have undergone the same rigorous development and testing process as other vaccines, but over a shorter time period. A number have been approved as safe to use.



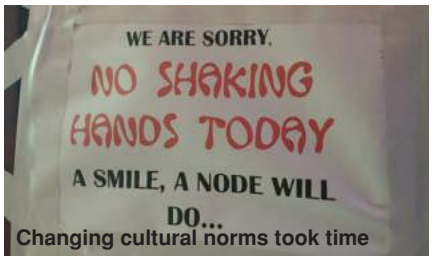
4. Explain how the Covid-19 vaccines were developed in a much shorter time than most other vaccines:

Controlling the 2014 Ebola outbreak: culture and medicine clash

- The distrust of the authorities, Western culture, and a lack of education around medical practices and hygiene in many parts of the world, create barriers to controlling the outbreak of disease. The 2014-2016 Ebola outbreak in West Africa highlighted these issues.
- Ebola spreads by contact with body fluids. To reduce its spread, standard practice is to limit touching such as hugging and hand shakes, and restrict access to the sick or dead. In a culture where assisting the sick and honouring the dead is an important part of family life, the imposing of these restrictions can simply increase distrust of the authorities.
- Medical authorities faced suspicion that doctors were actually spreading the virus; riots broke out while market places were being disinfected, equipment was stolen, and facilities were attacked. Several healthcare workers were killed while trying to educate the public about Ebola.
- The outbreak was controlled using numerous methods including surveillance and contact tracing, educating the public, providing facilities for hand washing, and disinfecting the dead to reduce transmission to living relatives. Involving community leaders and clergy in public education around treatment of the dead and burials was an important part of reducing Ebola's spread.


People found doctors in hazmat suits intimidating

CDC



Changing cultural norms took time

CDC

5. Why is it important for medical authorities during a disease outbreak to understand the cultures they are working with, and educate people about what they are trying to accomplish?

ISBN: 978-1-98-856655-9

189 Aboriginal Protocols in Medicine

Key Idea: Traditional knowledge and property rights need to be acknowledged when developing traditional medicines for commercial use.

Indigenous Australian people have long used native plants for traditional medicines. Most modern medicinal drugs are manufactured industrially on a vast scale and the majority are based on chemicals made naturally in living organisms. As more pathogens become resistant to current medicines, it is important that researchers explore the natural world for new chemicals that could potentially be used against these pathogens and possibly act as new treatments for cancer. However, the production and use of medicines derived from plants used in traditional medicine raises many ethical and legal questions over who actually owns the knowledge.

- Australian flora is rich in plants that produce chemically active compounds such as aromatics, alkaloids, tannins, and oils. In the 50-60 thousand years that Aboriginal Australians have inhabited Australia, they have identified and used a wide range of plants that have medicinal value. The traditional use of these plants is commonly called bush medicine.

Mark Marathon CC 3.0

Gumbi gumbi (*Pittosporum angustifolium*) is traditionally used to treat coughs, colds, and even eczema. Research shows it may have use as an antiviral, and blood pressure regulator.

John Jennings CC 2.0

Tea tree (*Melaleuca*) oil is well known for its antiseptic properties. Vapour from crushed leaves can be used to treat headaches. Tea brewed from it helps relieve coughs and colds.

Geoff Derrin CC 4.0

The leaves of emu bush (*Eremophila longifolia*) have been used to treat wounds, headaches, and chest pains. Smoke from the leaves can create a sterile environment.

Smoke bush

- The smoke bush plant (*Conospermum*) is traditionally used in Aboriginal bush medicine. In 1960, specimens were collected by the US Cancer Institute and tested. No useful properties for use against cancer were found and the samples were put in storage. In the 1980s, following the spread of HIV, the samples were tested for use against the virus. The chemical, conocurovone, was found and shown to destroy HIV, in low concentrations.
- A patent was filed in the US in 1993 and in Australia in 1994. The patents gave the US Government the exclusive rights to developed compounds from the smokebush plant and to license them to other companies.
- Via the West Australian Government, commercial rights were licensed to AMRAD, an Australian pharmaceuticals company. No acknowledgement of traditional Aboriginal knowledge was included. There was also a possibility that the traditional knowledge holders could be excluded from using any sort of plant product.
- This is an example of what has been termed 'biopiracy', in which a company applies for a patent to a traditional resource that can stop any traditional use of the resource while making profit of their own.

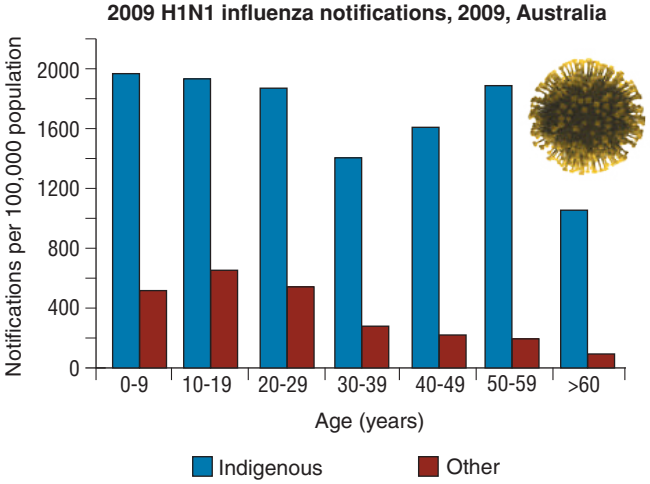
Casliber CC 3.0

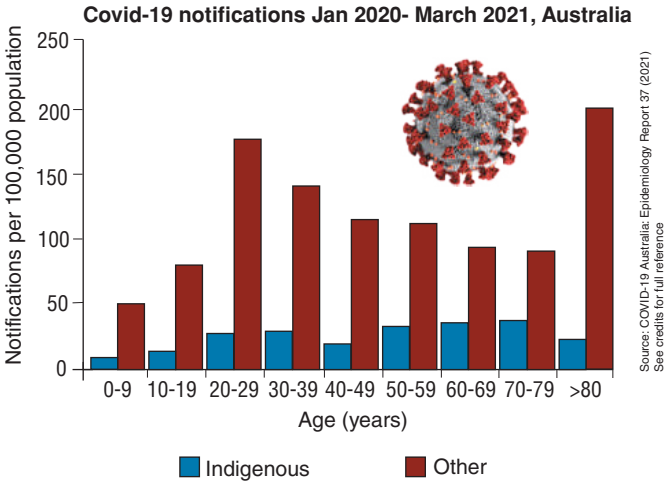
1. In groups of 3-4 investigate traditional Aboriginal medicines and identify ways to protect traditional knowledge and property rights. Useful links can be found in **BIOZONE's Resource Hub**. Report back to your class on a traditional medicine (not mentioned above) you have identified, if it has any possible commercial uses or development, and how the traditional knowledge and rights will be acknowledged or protected. Summarise the results of your investigation below:

ISBN: 978-1-98-856655-9

190 Chapter Review: Did You Get It?

1. The data below compares Australia's response to the 2009 outbreak of H1N1 influenza to early responses to the outbreak of Covid-19. In both cases, early efforts to delay the entry of the disease into Australia focused on border control. However, while Australia's borders were never closed for the 2009 H1N1 flu, they were closed quickly for the Covid-19 outbreak. Movement restrictions (within and between states) were also used, as necessary (e.g. following community transmission), to stop the spread of Covid-19. Many remote Indigenous communities isolated themselves from the greater populace.

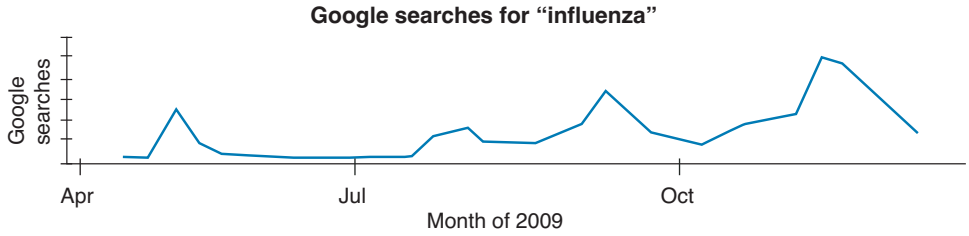




(a) Study the data above. How did the different responses in Australia to H1N1 and Covid-19 affect the way the diseases spread through the population?

(b) Why was the response to Covid-19 so different from the resonse to H1N1 flu?

2. Various health intelligence networks e.g. the Global Public Health Intelligence Network, monitor internet searches in order to determine if a disease outbreak is imminent. For example, the graph below shows the number of Google searches including the word 'influenza' for 2009.



How would monitoring the number of internet searches about diseases or symptoms help identify and locate potential outbreaks?

3. The omicron variant of Covid-19 has an R_0 value of between 8 and 15. What does this mean? What percentage of the population would need to be vaccinated to control the disease?

ISBN: 978-1-98-856655-9

191 Synoptic Assessment: Module 7

1. Over their lifetime, a person can develop resistance to specific pathogens. This is called acquired immunity. Acquired immunity can be obtained through natural or artificial means, and by active or passive processes.

   • Use examples to describe the differences between naturally and artificially acquired immunity
   • Discuss the differences and similarities between passive immunity and active immunity:

2. The table below shows the incidence of malaria from 2008 to 2018:

	Incidence of malaria (per 1,000 population at risk)						
Year	Africa Eastern and Southern	Africa Western and Central	East Asia & Pacific	Latin America & Caribbean	Middle East & North Africa	South Asia	Sub-Saharan Africa
2008	208	391	5	6	3	16	290
2009	211	384	6	5	4	16	288
2010	204	374	7	6	6	16	280
2011	189	360	5	5	4	14	265
2012	178	345	6	4	5	11	253
2013	168	330	5	4	4	9	240
2014	157	313	4	4	3	9	226
2015	162	298	3	4	3	9	222
2016	172	288	3	5	4	10	224
2017	176	285	4	7	4	7	225
2018	167	284	3	7	4	5	219

(a) What is the global trend in malaria incidence from 2008 to 2018?

(b) What area has consistently had the greatest incidence of Malaria?

(c) Identify factors that could be causing the reduction in global Malaria cases:

ISBN: 978-1-98-856655-9

3. (a) Identify a specific pathogen and describe one adaptation that facilitates its entry into a host:

(b) Pathogen A is transmitted by direct person to person contact (e.g. touch). Pathogen B is transmitted by aerosol (e.g. coughing). Explain which mode of transmission is likely to cause the greatest spread of a disease:

4. It is important to maintain high standards of hygiene in medical environments such as hospitals or aged care facilities, to prevent the spread of pathogens. Handwashing with soap and water reduces the number of bacteria present, but it is not always convenient or possible to do this. The use of alcohol-based sanitisers has become a common alternative.
The data (right) shows the effect of handwashing or alcohol sanitiser on reducing bacterial load on the fingers of a group of intensive care nurses. 204 samples were taken from the nurses' fingers to determine the base level of contamination (shown by growth of bacterial colonies on agar). The nurses were then split into two groups (soap or alcohol rub). After they had cleaned and dried their hands, the fingers were pressed onto agar to determine the remaining bacterial load.

The effect of hand wash versus alcoholic hand rub on the disinfection of hands.

Bacterial growth on agar plates	Untreated hands (*n* = 204)	After soap & water wash (*n* =102)	After alcohol rub (*n* =102)
No growth or scanty growth (< 20 colonies)	16	51	91
Moderate growth (20-100 colonies)	136	44	5
Heavy growth (> 100 colonies)	52	7	0

Data source: Mallekal, M et.al (2005) Indian J Crit Care Med. Vol 9(3).

(a) Did the two treatments reduce bacterial contamination?

(b) Which treatment was most effective?

(c) What evidence supports your choice?

5. Hong Kong is a densely packed region, with 7 million people within 1,104 km^2. In 1997, there was an outbreak of avian influenzavirus (bird flu) in Hong Kong. All 18 humans infected had been in recent contact with live domestic fowl (e.g. chickens) in markets. Six of the 18 infected people died. Authorities ordered the slaughter of all live chickens within Hong Kong (1.6 million birds) and stopped the import of more birds. No further cases of bird flu in humans were reported.

(a) Health authorities suspected that the chickens were the source of the virus. Based on the information above decide if you think they were correct in their hypothesis and explain your reasoning:

(b) Based on the evidence, do you think that the large scale slaughter of chickens and the ban on bird imports were justified? Explain your reasoning:

ISBN: 978-1-98-856655-9

MODULE

08

Non-infectious Disease and Disorders

Student outcomes:

- Analyse and evaluate primary and secondary data and information
- Solve scientific problems using primary and secondary data, critical thinking skills, and scientific processes
- Communicate scientific understanding using suitable language and terminology
- Explain non-infectious disease and disorders, and describe a range of technologies and methods used to assist, control, prevent, and treat non-infectious diseases

CHAPTER 13

Homeostasis

Key terms

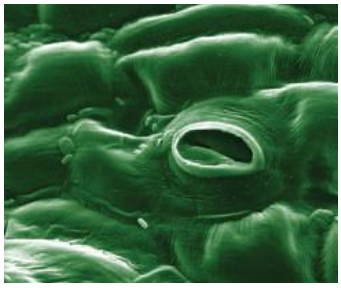
alpha cells
antagonistic
beta cells
control centre
effector
endocrine
endotherm
glucagon
glucose
glycogen
homeostasis
hormone
hypothalamus
insulin
liver
negative feedback loop
neuron
osmosis
pancreas
pituitary gland
receptor
response
stimulus
stomata
thermoreceptor
thermoregulation
thyroid gland
transpiration
type 1 diabetes
water potential

Inquiry question: *How is an organism's internal environment maintained in response to a changing external environment?*

Activity number

Negative feedback loops

Key skills and knowledge



Mechanisms for maintenance of internal environment

Key skills and knowledge

192 Homeostasis

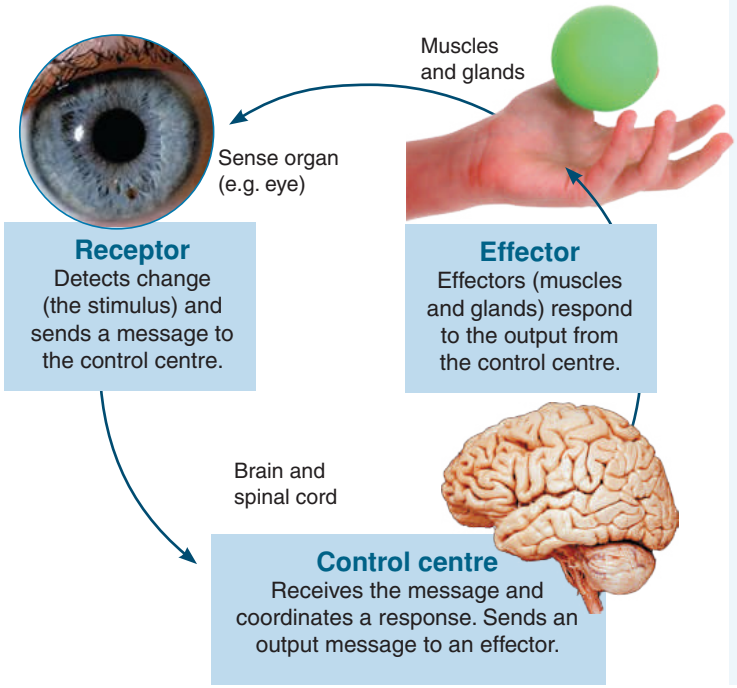
Key Idea: Homeostasis is the process of sustaining a constant physiological state within the body, regardless of fluctuations in the external environment.

Organisms maintain a relatively constant physiological state, called **homeostasis**, despite changes in their environment. Any change in the environment to which an organism responds is called a **stimulus**. Environmental stimuli are constantly changing, so organisms must adjust their behaviour and physiology constantly to maintain homeostasis. This requires the coordinated activity of the body's organ systems. Homeostatic mechanisms prevent potentially harmful deviations from the steady state, and keep the body's **internal conditions** within strict limits.

Homeostasis is required to maintain constant body temperature, at about 37°C. Similarly, you must regulate blood sugar (glucose) levels and blood pH, water and electrolyte balance, and blood pressure. Your body's organ systems coordinate to carry out these tasks.

How homeostasis is maintained: the stimulus-response model

To maintain homeostasis, the body must detect stimuli through **receptors**, process this sensory information in a **control centre**, and respond to it appropriately via an **effector.** The responses provide new feedback to the receptor. These three components are illustrated below.



Homeostasis analogies

The analogy of a temperature setting on a heat pump is a good way to explain how homeostasis is maintained. A heat pump has sensors (a receptor) to monitor room temperature. It also has a control centre to receive and process the data from the sensors. Depending on the data it receives, the control centre activates the effector (heating/cooling unit), switching either on or off.

When the room is too cold, the heating unit switches on, and the cooling unit is off. When it is too hot, the heating unit switches off and the cooling unit is switched on. This system maintains a constant temperature, similar to homeostasis in the body.

The analogy of staying upright on a mountain bike, using body weight, arms, pedals, brakes, and steering, demonstrates that many homeostasis systems have multiple mechanisms to maintain a steady state.

1. Define the term, homeostasis: _____

_____

2. What is the role of the following components in maintaining homeostasis?

   (a) Receptor: _____

   _____

   (b) Control centre: _____

   _____

   (c) Effector: _____

   _____

ISBN: 978-1-98-856655-9

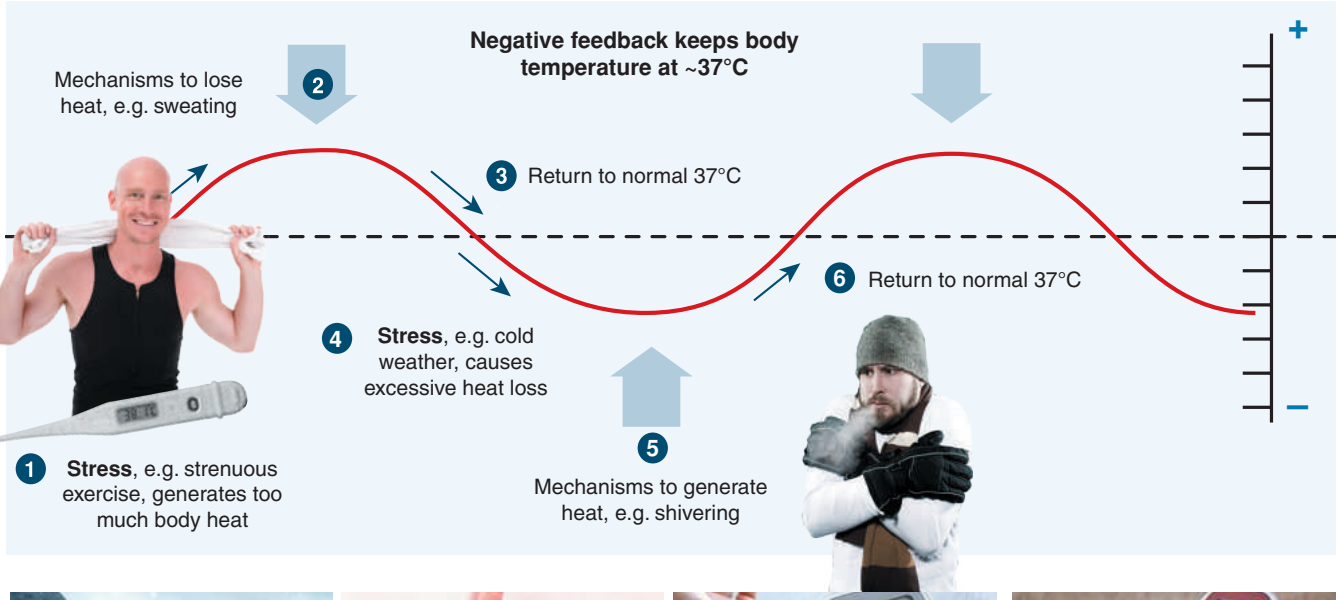
193 Feedback Loops

Key Idea: Feedback loops, driven by various mechanisms, can stabilise biological systems or exaggerate deviations from the median condition.

Two types of feedback loop are used in the body, each producing specific outcomes. **Negative feedback loops** maintain homeostasis, e.g. regulation of body temperature. **Positive feedback loops** exaggerate any changes in the internal environment, moving the body away from a stable state by quickly amplifying changes in the internal environment e.g. blood clotting.

Negative feedback loops

- Negative feedback loops are control systems that maintain the body's internal environment at a relatively steady state.
- When variations from the norm are detected by the body's receptors, a response or output from the effectors that opposes the stimulus is classified as negative feedback.
- Negative feedback discourages variations from a set point and returns internal conditions to a steady state.
- Most physiological systems achieve homeostasis through negative feedback loops.



We know when we are cold. Although we are unaware of most of the negative feedback loops operating in our bodies, they keeps our systems stable.

Food in the stomach activates stretch receptors, stimulating gastric secretion and motility. As the stomach empties, the stimulus for gastric activity declines.

Negative feedback loops control almost all the body's functioning processes, including heart rate, blood glucose, blood pressure, and pituitary secretions.

Maintaining a stable blood glucose level is an important homeostatic function regulated by negative feedback. It involves two antagonistic hormones.

1. Construct a diagram of a negative feedback loop that models **thermoregulation** (homeostasis of constant body temperature) using information and terms in this activity:

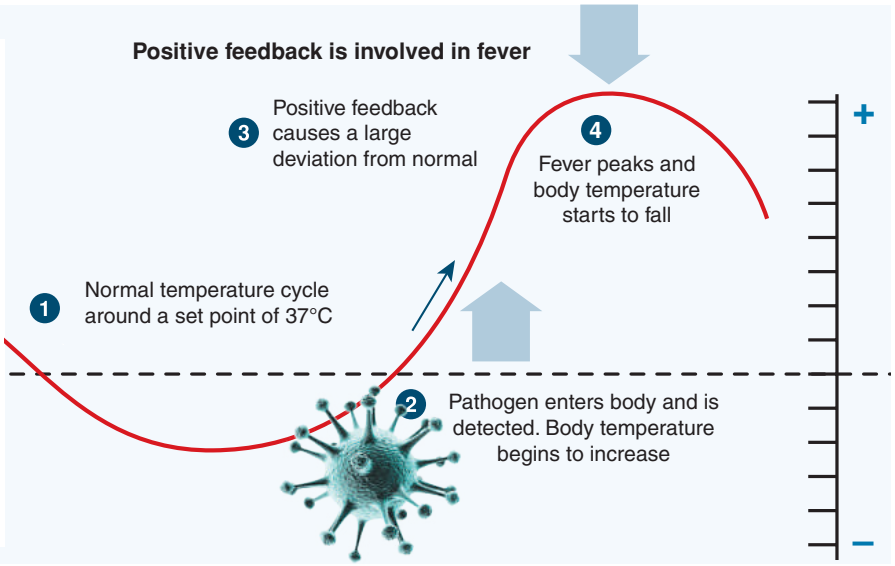
ISBN: 978-1-98-856655-9

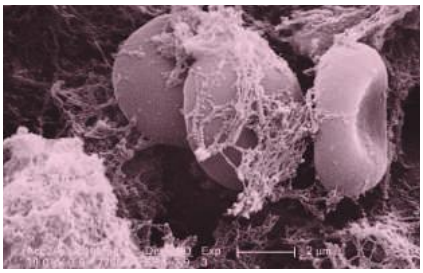
Positive feedback loops

- Positive feedback loops amplify (increase) a response in order to achieve a particular result. Examples include fruit ripening, fever, blood clotting, childbirth (labour), and lactation (production of milk).
- The mechanisms within a positive feedback loop will cease to function once the end result is achieved (e.g. the baby is born, a pathogen is destroyed by a fever, or ripe fruit falls from a tree). Positive feedback loops are less common than negative feedback loops in biological systems because the escalation in response is unstable. Unresolved positive feedback responses (e.g. high fevers) can be fatal.

Fever

Infection can reset normal temperature control so that body temperature increases above the normal range, resulting in a fever. Fever is an important defence against infection.





CDC

Positive feedback is involved in blood clotting. A wound releases chemicals that activate platelets in the blood. Activated platelets release chemicals that activate more platelets, forming a blood clot.

Ragesoss

Ethylene is a gaseous plant hormone involved in fruit ripening. It accelerates ripening in nearby fruits so that these also ripen, releasing more ethylene. Too much ethylene causes over-ripening.

Childbirth involves positive feedback. Pressure of the baby's head causes release of a hormone that increases contractions. The feedback loop ends when the baby is born.

2. (a) Why are positive feedback loops much less common than negative feedback loops in body systems? _____

_____

(b) How can positive feedback lead to a runaway response in the body? _____

_____

_____

(c) Why can positive feedback be dangerous if it continues for too long? _____

_____

_____

(d) How is a positive feedback loop normally stopped? _____

_____

(e) Predict what could happen if a person's temperature continued to increase during a fever, i.e. did not peak, then fall:

_____

_____

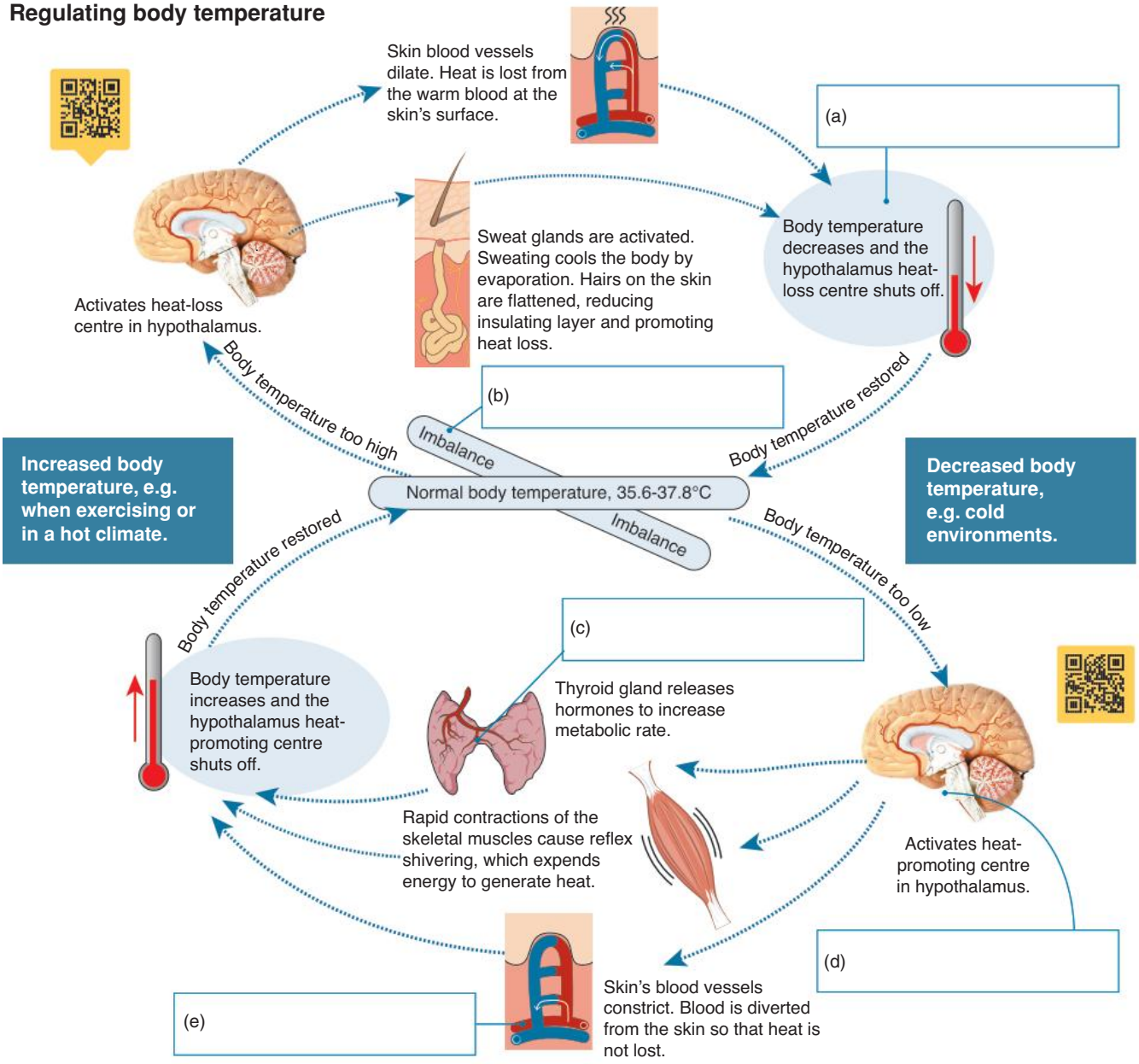
ISBN: 978-1-98-856655-9

194 Mechanisms of Thermoregulation

Key Idea: The hypothalamus acts as the control centre in the thermoregulation negative feedback loop.

In humans, the temperature regulation centre is a region of the brain called the **hypothalamus**. It contains thermoreceptors that monitor core body temperature and has a 'set-point' temperature of 36.7°C. The hypothalamus acts like a thermostat. It registers changes in the core body temperature and also receives information about temperature changes from thermoreceptors in the skin. It then coordinates effector responses to counteract the changes and restore normal body temperature. Communication between components of the negative feedback loop uses both nervous and hormonal pathways. When normal body temperature is restored, the corrective mechanisms are switched off.

Regulating body temperature



1. In the diagram above showing the regulation of body temperature:

   (a) Identify the stimulus: _____

   (b) Identify the effectors: _____

   (c) What structure(s) would you add to represent the receptors? _____

2. Label the diagram above by appropriately adding the labels: stimulus, receptors, control centre, and effectors.

3. How do the effectors restore body temperature when it increases above the set point? _____

   _____

   _____

ISBN: 978-1-98-856655-9

Thermoreceptors

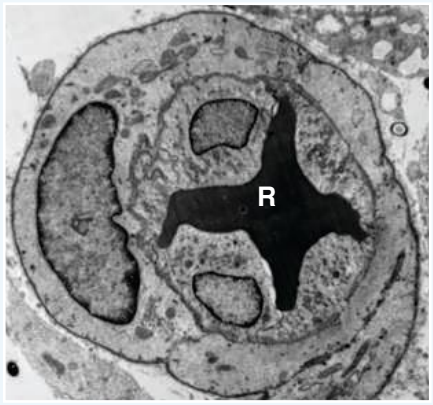
- **Thermoreceptors** are simple sensory receptors located both inside the body and embedded in the skin. They respond to changes in temperature. When they detect a temperature change they send information (as nerve impulses) to the hypothalamus. Heat thermoreceptors detect an increase in skin temperature above 37.5°C and cold thermoreceptors detect a fall below 35.8°C.
- The hair erector muscles, sweat glands, muscles, and blood vessels are the effectors for mediating a response to information from thermoreceptors.
- The temperature changes resulting from the actions of the effectors is communicated as negative feedback to the thermoreceptors, and relayed back to the hypothalamus to deactivate once normal body temperature is reached. Illness can 'set' the temperature higher, felt as a fever.

Vasodilation and sweating are mechanisms in response to high temperature or exertion.

Ildar Sagdejev CC 3.0

Vasoconstriction and 'goosebumps' (piloerection) are in response to low temperature or inactivity.

Regulating blood flow to the skin



RM Hunt CC 3.0

Constriction of a small blood vessel. A red blood cell (R) is in the vessel (TEM).

To regulate heat loss or gain from the skin, the blood vessels underneath become narrower (vasoconstriction) to reduce blood flow, or expand (vasodilation) to increase blood flow. When blood vessels are fully constricted there may be as much as a 10°C gradient from the outer to the inner layers of the skin. Extremities such the hands and feet have additional vascular controls, which can reduce blood flow to them in times of severe cooling.

4. Describe the role of each of the following in regulating body temperature:

   (a) Shivering: _____

   _____

   (b) The skin: _____

   _____

   (c) Nervous input to effectors: _____

   _____

   (d) Hormones: _____

   _____

5. What is the purpose of sweating and how does it achieve its effect? _____

   _____

   _____

6. Explain how negative feedback is involved in the regulation of body temperature: _____

   _____

   _____

   _____

   _____

7. How do the blood vessels help to regulate the amount of heat lost from the skin and body? _____

   _____

   _____

ISBN: 978-1-98-856655-9

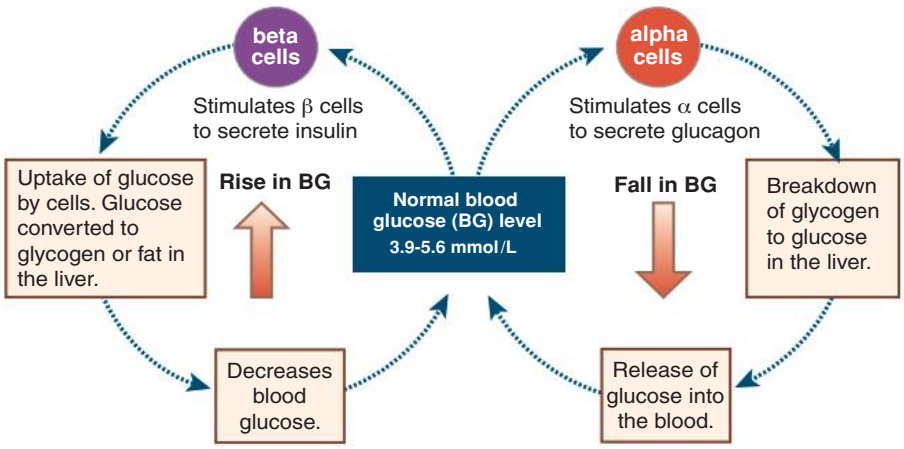
195 Control of Blood Glucose

Key Idea: Insulin and glucagon are the two hormones, secreted by the pancreas, that maintain blood glucose at a steady state via a negative feedback loop.

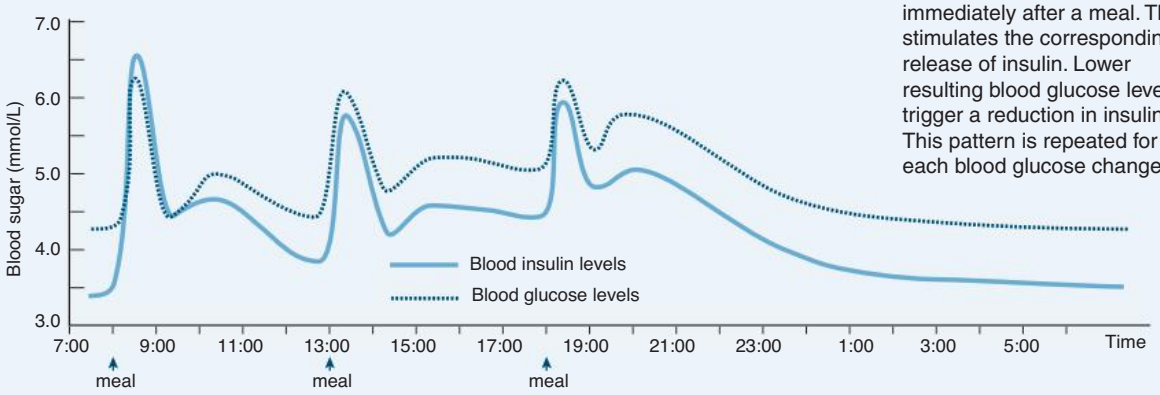
Insulin and glucagon are produced by the islet cells of the pancreas and control blood glucose levels. Insulin lowers blood glucose by promoting the uptake of glucose by the body's cells and the conversion of glucose into the storage molecule glycogen in the liver. Glucagon increases blood glucose by stimulating the breakdown of stored glycogen and the synthesis of glucose from amino acids. The liver has a central role in these carbohydrate conversions. Negative feedback stops hormone secretion when normal blood glucose levels are restored. Blood glucose homeostasis allows energy to be available to cells, as required.

Blood glucose can be tested using a finger prick test. The glucose in the blood reacts with an enzyme electrode, generating an electric charge proportional to the glucose concentration. This is displayed as a digital readout.

Negative feedback in blood glucose regulation



Blood glucose and insulin levels



Blood glucose levels rise immediately after a meal. This stimulates the corresponding release of insulin. Lower resulting blood glucose levels trigger a reduction in insulin. This pattern is repeated for each blood glucose change.

1. (a) Identify the stimulus for the release of insulin: _____

   (b) Identify the stimulus for the release of glucagon: _____

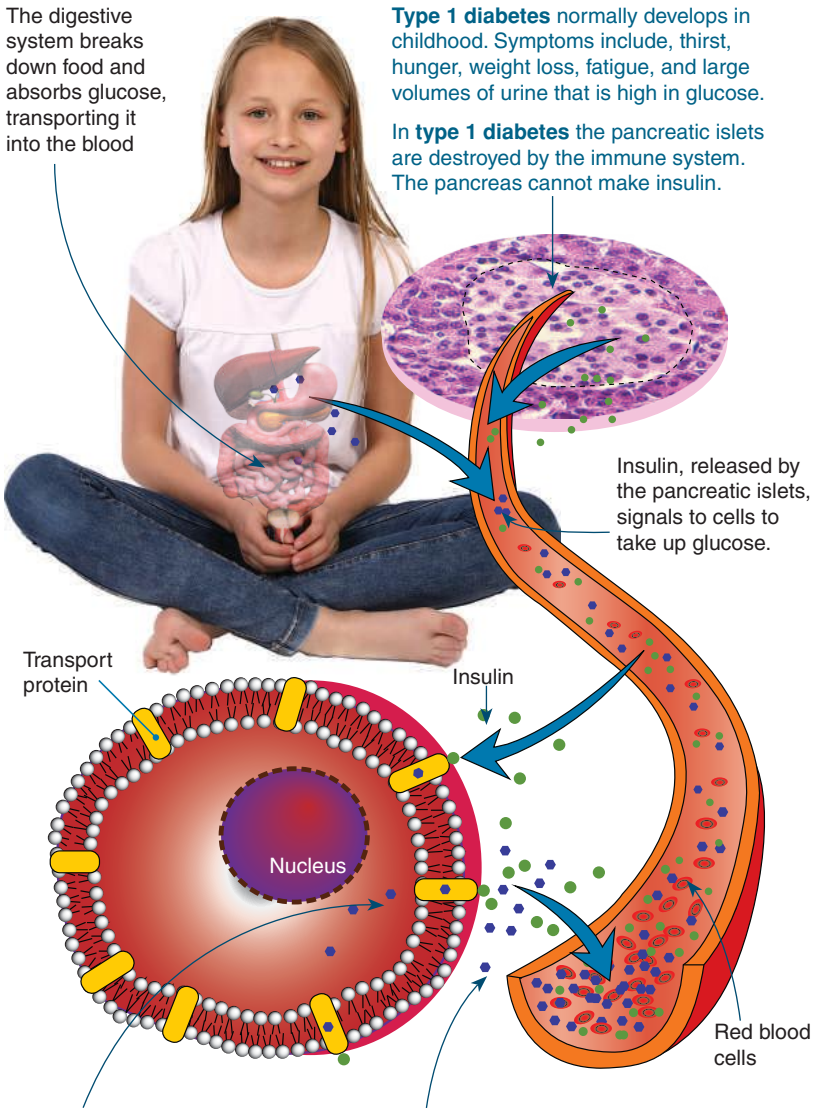
2. Use the table to compare and contrast the homeostasis of temperature regulation and blood glucose control:

Negative feedback loop	Differences	Similarities
Thermoregulation	(a)	(c)
Blood glucose control	(b)	

ISBN: 978-1-98-856655-9

Type 1 diabetes

Diabetes mellitus (often called diabetes) is a condition in which blood glucose is too high because the body's cells cannot maintain homeostasis of glucose in the normal way. Diabetes mellitus is characterised by large volumes of sweet urine and extreme thirst. In **type 1 diabetes**, the insulin-producing beta cells of the pancreas are destroyed and no insulin is produced. Patients must have regular insulin injections to stabilise blood glucose levels.



Short term effects

Low blood sugar *(hypoglycaemia)*: Blood glucose levels below normal (<4 mmol/L) can result in clumsiness, confusion, and seizures. It can be caused from too much insulin, usually after injection, if glucose levels are already low.

High blood sugar *(hyperglycaemia)*: High blood glucose levels (> 6 mmol/L) occur when glucose fails to enter the cells. Effects include frequent urination, fatigue, thirst, and blurred vision.

Ketoacidosis: A lack of insulin can result in a build up of molecules called ketones caused by metabolism of fats for fuel. Ketones are acidic and can lead to metabolic acidosis (fall in tissue pH), which can rapidly be fatal.

Long term effects

General circulation: Over time, high blood glucose damages the lining of small blood vessels, making them prone to developing plaques and becoming narrow and clogged. The result of this is increased blood pressure.

Heart disease: Nearly 3 in 4 people with type 1 diabetes will suffer some form of heart disease. Causes may be from autoimmune responses, and high blood glucose and blood pressure.

Kidney disease: Damage to the small blood vessels of the kidney causes kidney function to decline and produces many associated health problems. Glucose in the urine can also result in fungal infections in the bladder.

Eye problems: Damage to the blood vessels in the eyes leads to cataracts and retinal damage.

Nerve damage: High blood glucose levels cause nerve damage indirectly, through blood vessel damage. Symptoms include tingling and weakness in the limbs. Numbness can lead to unnoticed and hard to treat infections and ulcers.

3. (a) What is type 1 diabetes? _____

   (b) Explain how the usual negative feedback loop for blood glucose homeostasis is disrupted in a person with type 1 diabetes. How does this disruption cause the symptoms observed?

   _____

   _____

   _____

   _____

4. How do regular insulin injections help a person with type 1 diabetes to maintain their blood glucose homeostasis?

   _____

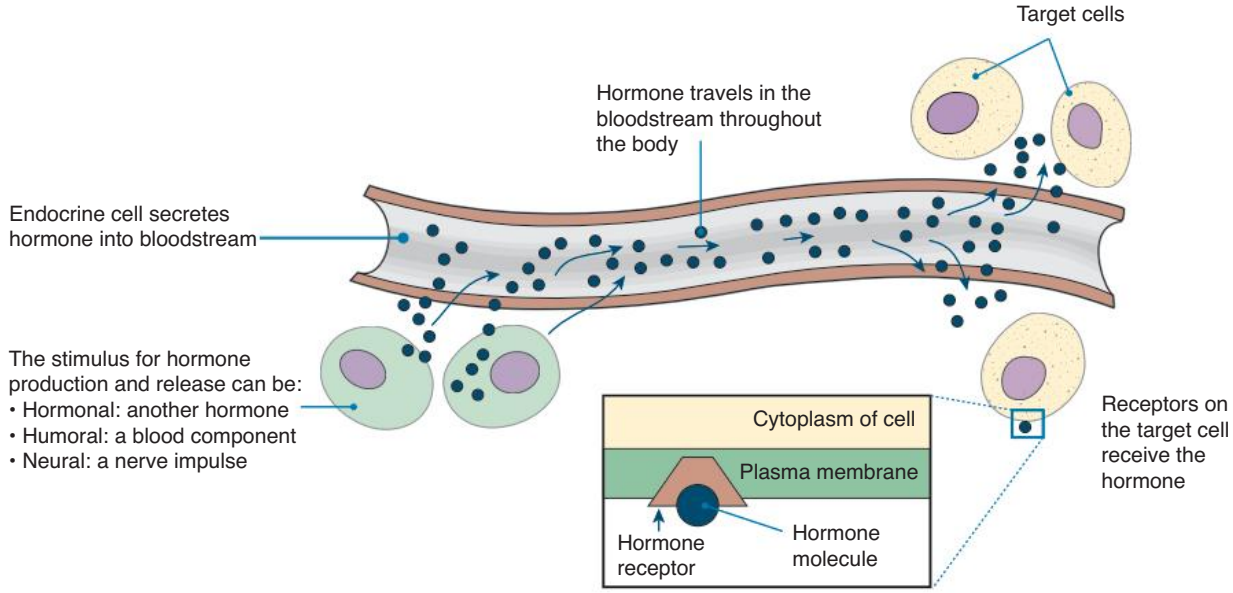
   _____

ISBN: 978-1-98-856655-9

196 Hormonal Regulation

Key Idea: The endocrine system is an internal coordination system that is involved in maintaining homeostasis.

In mammals, the two main internal coordination systems that maintain homeostasis are the endocrine system and the nervous system. They provide a pathway from stimulus to response, either acting independently or together. The two systems are quite different in their modes of action, the responses they elicit, and the duration of action. The endocrine system produces a slower, more long-lasting response through blood-borne chemicals called hormones. The hormonal pathway starts with endocrine cells or glands secreting a hormone directly into the bloodstream in response to a particular stimulus. It ends when target cells receive the hormone and action a response.



How hormones work

- The endocrine system is made up of endocrine cells (organised into endocrine glands) and the hormones they produce. Endocrine glands secrete hormones directly into the bloodstream, targeting specific receptors located on certain cells or tissues in the body. The type and strength of the stimulus determines which specific hormone is released, and therefore what response is elicited as a result.
- Although hormones are distributed throughout the body via the network of blood vessels, they affect only specific target cells. These target cells have receptors on the plasma membrane which recognise and bind the hormone. The binding of the hormone and receptor triggers the response in the target cell. Cells are unresponsive to a hormone if they lack the appropriate receptors.

Types of stimulus for hormone production		
Hormonal stimuli	**Humoral stimuli**	**Neural stimuli**
The release of one hormone can stimulate the release of another, with sometimes more than two steps involved in the process. The pituitary gland and hypothalamus often play a role in these types of stimuli.	Changes in the concentration of a substance or ion in the blood can act as a stimulus in some negative feedback loops.	Hormone producing glands can be directly stimulated by a neural impulse. Stress and 'fight-or-flight' responses are common examples of this type of stimulus.

1. (a) What is a hormone? _____

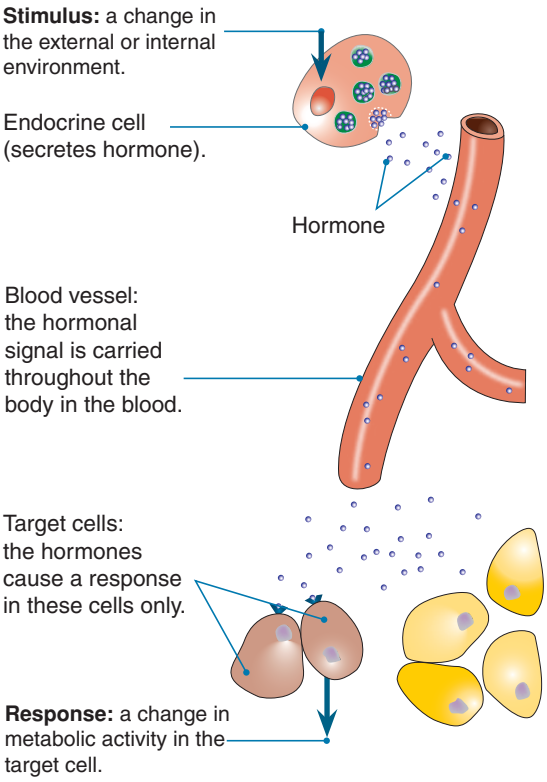
(b) Why can a hormone only influence specific target cells even though all cells may be exposed to the hormone?

(c) What is the stimulus for the release of insulin: humoral, hormonal, or neural? _____

2. The endocrine system consists of glands, yet the sweat glands are not considered part of the endocrine system. What two features are required for a gland to be classified as part of the endocrine system?

ISBN: 978-1-98-856655-9

Hormonal pathways in an endocrine system

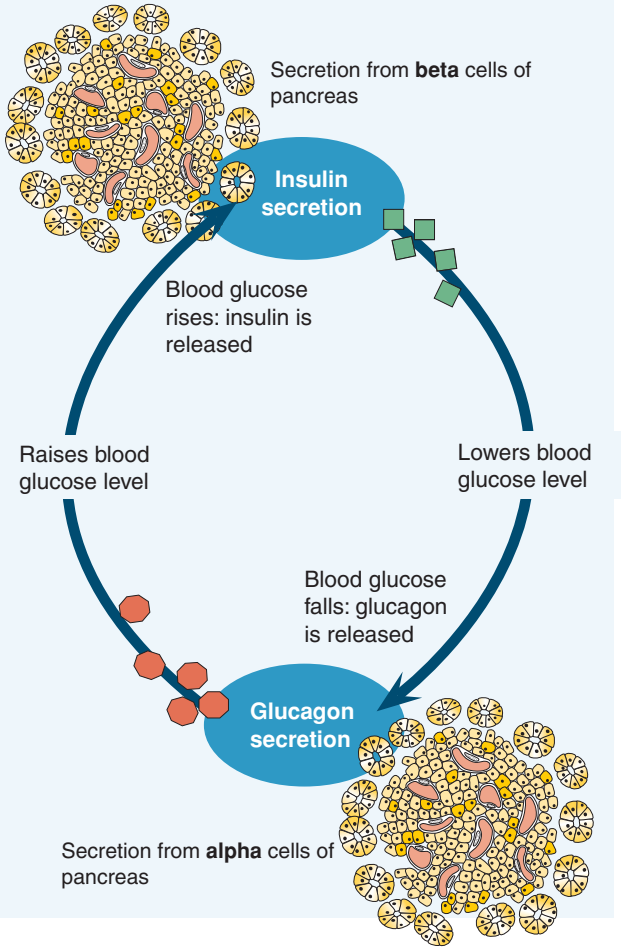


How hormones work	
Communication	Hormones in the blood
Speed	Relatively slow (over minutes, hours, or longer)
Duration	Longer lasting effects
Target pathway	Hormones broadcast to target cells everywhere
Action	Causes changes in metabolic activity

Antagonistic hormones in blood glucose control

A pair of hormonal pathways can operate together to maintain homeostasis, with the feedback mechanism used to adjust the balance. The effects of one hormone are often counteracted by an opposing hormone. They are therefore referred to as antagonistic pairs.

Example: Insulin acts to decrease blood glucose and glucagon acts to raise it.



3. Complete the flowchart, summarising key steps in a hormonal pathway during a homeostasis response:

Key step: (a)	→	Key step: (b)	→	Key step: (c)

4. Explain how antagonistic hormones act to maintain homeostasis: _____

_____

5. What function do the following organs have in the hormonal regulation of blood glucose control?
   (a) The pancreas: _____

_____

_____

   (b) The liver: _____

_____

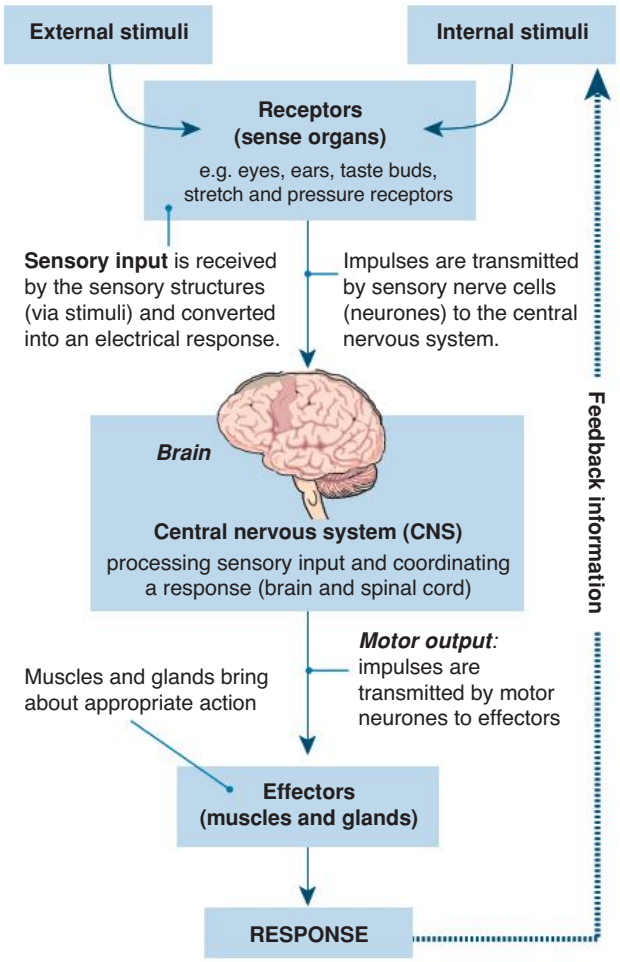
ISBN: 978-1-98-856655-9

197 Nervous Regulation in Vertebrates

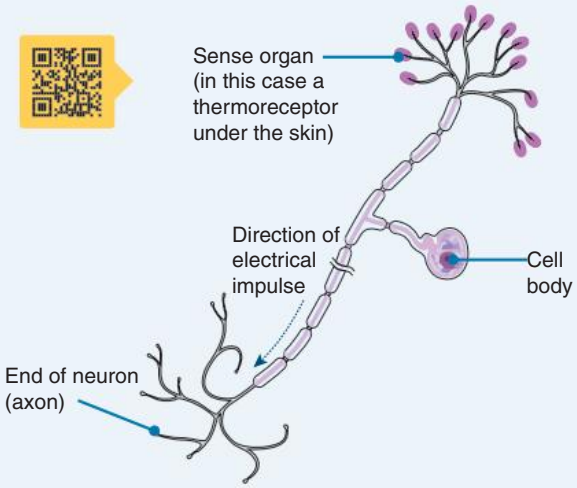
Key Idea: The neurons of the nervous system transmit information as nerve impulses, to provide rapid responses to stimuli that affect the stability of the body.

The nervous system plays a key role as another main internal coordination system involved in maintaining homeostasis, and acts as a signalling network. In response to stimuli, rapid and short-lived electrical signals are transmitted directly between adjacent **neurons** and across the synapse gap between them with neurotransmitters. Impulses can be transmitted over considerable distances and the response is precise and rapid. Sense organs, or nerve cell endings, detect stimuli and initiate signals to the central nervous system to coordinate a response. Further signals are then transmitted to muscles or glands acting as effectors.

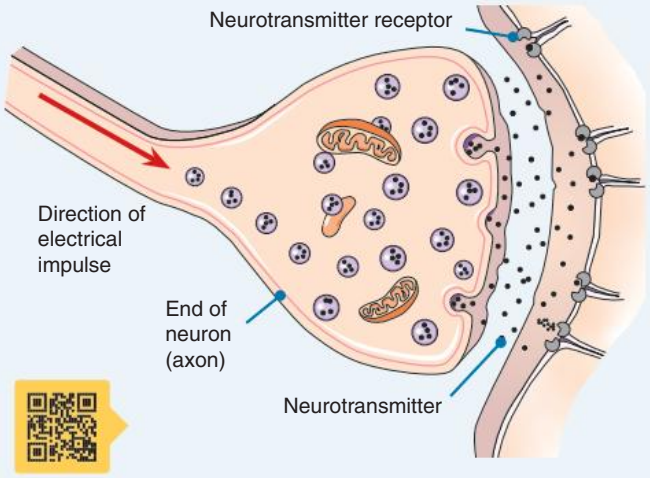
Coordination by the nervous system



Close-up view of a sensory neuron



Close-up view of a synapse



- The vertebrate nervous system consists of the brain and spinal cord that, collectively, form the central nervous system. This system is connected to nerves and receptors, known as the peripheral nervous system. Sensory input to receptors comes via stimuli. Information about the effect of a response is provided by feedback mechanisms so that the system can be readjusted. The basic organisation of the nervous system can be simplified into a few key components: the sensory receptors, a central nervous system processing point, and the effectors, which bring about the response.
- Neural communication is important in rapid responses to stimuli, and may also work together with the endocrine system to trigger the release of hormones.

1. Identify the three basic components of a nervous system and describe their role:

   (a) _____

   (b) _____

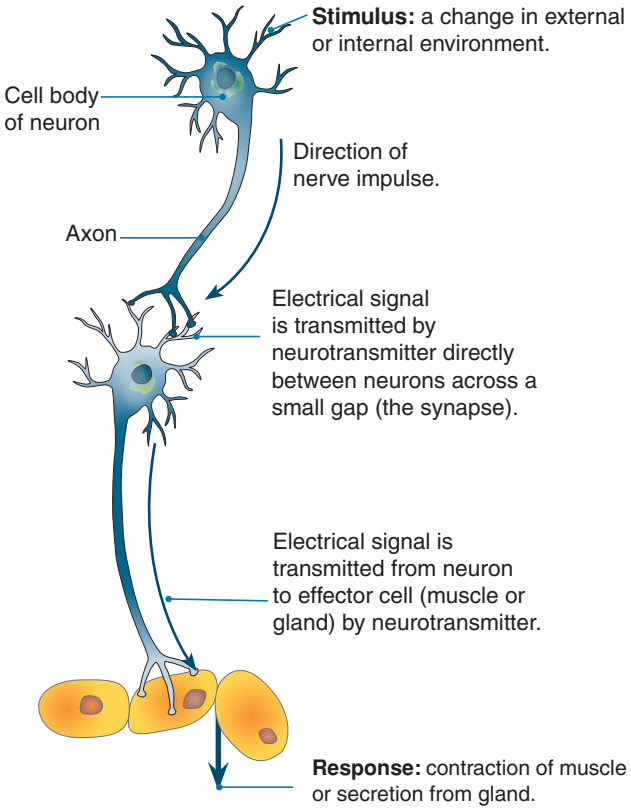
   (c) _____

ISBN: 978-1-98-856655-9

Signalling pathway of the nervous system

The vertebrate nervous system is able to detect external stimuli using sense organs containing specialised sensory cells such as those found in the eye, or taste buds in the mouth. For detection of internal stimuli, such as body temperature, the receptors are often much simpler nerve endings. Once stimuli are received, an electrical impulse is sent along one or more neurons. If the signal is large enough it transfers across the synapse gap between neurons using the movement of substances, known as neurotransmitters. Effector cells, forming either muscles or secretory glands, also use neurotransmitters to receive a signal from adjoining neurons, which then initiates a response.

Neural pathways



How neural control works	
Communication	Impulses across synapses
Speed	Very rapid (within a few milliseconds)
Duration	Short term and reversible
Target pathway	Specific (through nerves) to specific cells
Action	Causes glands to secrete or muscles to contract

In the example above, the frisbee's approach is perceived by the eye. The central nervous system processes the sensory message. Coordination of hand and body orientation is brought about via messaging through motor neurons to the muscles.

2. Describe the difference between external and internal environmental stimuli in thermoregulation: _____

3. Comment on the significance of the differences between the speed and duration of neural and hormonal pathways:

4. Complete the flowchart, summarising key steps in a neural pathway during a homeostasis response.

Key step: (a) → Key step: (b) → Key step: (c)

ISBN: 978-1-98-856655-9

198 Trends and Patterns in Endotherms

Key Idea: Endotherms regulate their body temperature to within narrow limits by controlling heat exchange with the environment and by generating heat from metabolism.

Heat exchanges with the environment occur via conduction, radiation, and evaporation. To maintain a relatively constant body temperature, endotherms must balance heat losses and gains. Thermoregulation is achieved through a variety of mechanisms: structural (physical attributes of the body); behavioural (the way an organism behaves); and physiological (mechanisms at the metabolic level). These mechanisms allow an organism to maintain a body temperature that is optimum for functioning. For endotherms that generate their body heat through metabolism, thermoregulation represents a high energy cost.

Thermoregulation mechanisms to prevent heat loss

The fairy penguin can be found in cold water and surrounding coastal areas around southern regions of Australia.

Physiological: Involuntary shivering when cooler core body temperature activates muscles.

Structural: No external ear flaps.

Behavioural: Feather fluffing to increase insulation layer when on land.

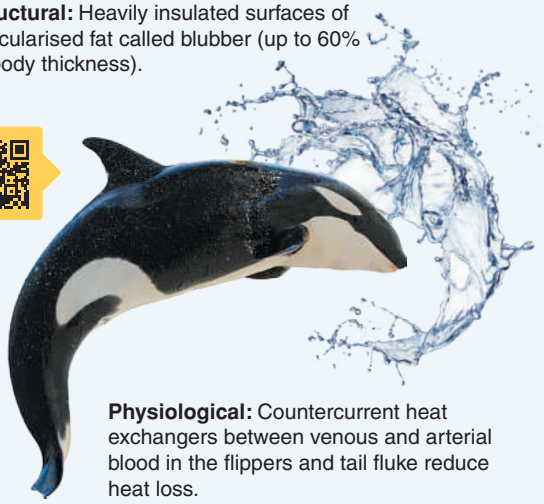
Structural: Overlapping feathers form waterproof layer to aid insulation.

Behavioural: Wings and legs held close to body.

Structural: Compact body shape with short legs.

The orca is found in oceans world-wide, including around many coastal areas of Australia.

Structural: Heavily insulated surfaces of vascularised fat called blubber (up to 60% of body thickness).



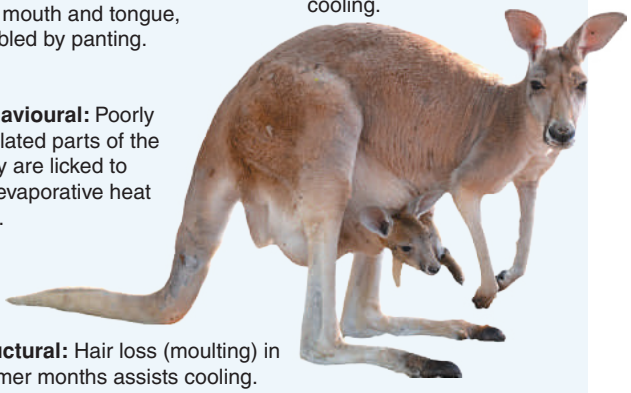
Physiological: Countercurrent heat exchangers between venous and arterial blood in the flippers and tail fluke reduce heat loss.

Thermoregulation mechanisms to increase heat loss

The red kangaroo lives in hot, dry desert and grassland areas of Australia.

Physiological: Evaporation from vascular rich mouth and tongue, enabled by panting.

Structural: Ears with large surface area aid cooling.



Behavioural: Poorly insulated parts of the body are licked to aid evaporative heat loss.

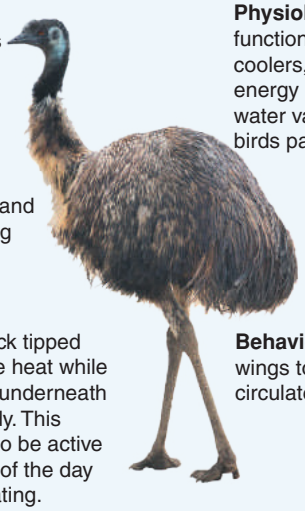
Structural: Hair loss (moulting) in warmer months assists cooling.

Kangaroos cannot sweat. Sweating cools by evaporation. Animals tend to rely on panting or sweating for cooling, not both.

The emu lives in both open dry plains and tropical forest areas of Australia.

Structural: Sparse feathers on the neck aid heat loss.

Physiological: Lungs function as evaporative coolers, removing heat energy into exhaled water vapour when the birds pant.



Behavioural: Shade seeking and swimming during hot days.

Structural: Black tipped feathers capture heat while lighter feathers underneath insulate the body. This allows the bird to be active in the hot parts of the day without overheating.

Behavioural: Stretches wings to allow air to circulate around body.

1. Endotherms use a number of different mechanisms to thermoregulate, although not all mechanisms are present in each species. Complete the table to summarise the key trends and patterns seen in thermoregulation mechanisms.

Mechanism	Preventing heat loss	Increasing heat loss
Structural	(a)	
Physiological	(b)	
Behavioural	(c)	

ISBN: 978-1-98-856655-9

199 Hormonal Mechanisms for Thermoregulation

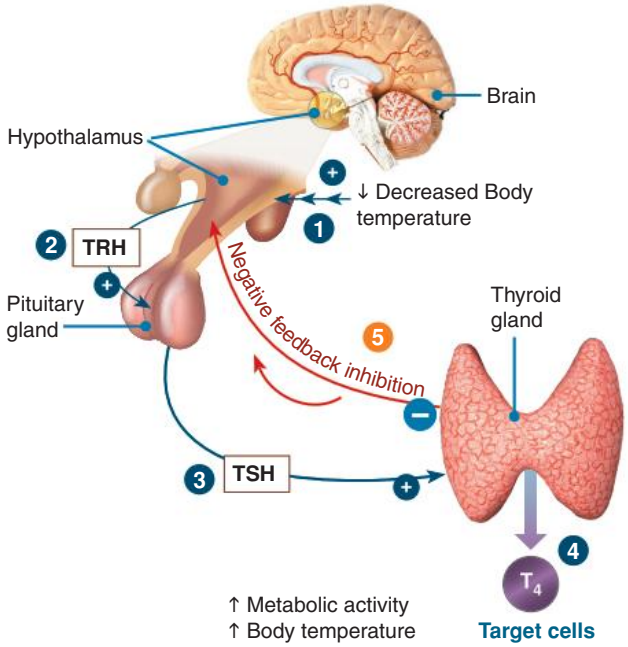
Key Idea: The hypothalamus, located on the underside of the brain, functions as a link between the endocrine and nervous internal coordination systems to maintain homeostasis.

The hypothalamus acts as a control system for hormone release by other glands throughout the body. When the nervous system registers a decrease in core body temperature, a signal is sent to the hypothalamus. This initiates a chain of hormone releases, via the pituitary gland, to reach the thyroid gland, situated under the larynx. Thyroxine, produced by the thyroid gland, is an important hormone in thermoregulation and causes an increase in internal heat by boosting metabolism. Insulin has also been shown to exert a thermoregulatory role and can have a direct effect on core body temperature.

Hormonal thermoregulation

Thyroxine (T_4) production is controlled by a negative feedback loop (shown below). This mechanism involves two parts of the brain, the hypothalamus and the pituitary gland.

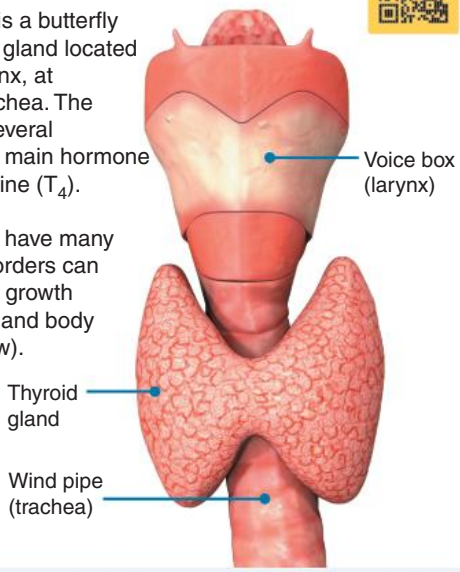
1. Low body temperature →
2. stimulates the hypothalamus to secrete thyrotropin releasing hormone (TRH) →
3. which in turn stimulates cells in the anterior pituitary to secrete thyroid stimulating hormone (TSH) →
4. TSH acts on the thyroid gland, causing it to produce thyroid hormones, including T_4 (thyroxine). T_4 binds to target cells, increasing their metabolic activity and producing heat. →
5. High levels of circulating thyroid hormones inhibit production of TRH and TSH. As a result, thyroid secretion is reduced.



The thyroid gland

The thyroid gland is a butterfly shaped endocrine gland located just below the larynx, at the front of the trachea. The thyroid secretes several hormones, but the main hormone produced is thyroxine (T_4).

Thyroid hormones have many functions, and disorders can affect metabolism, growth and development, and body temperature (below).



Graves' disease

The most common cause of hyperthyroidism is Graves' disease, characterised by an enlarged thyroid (goitre) and bulging eyes. In Graves' disease, the negative feedback loop is bypassed because a protein called thyroid stimulating immunoglobulin (TSI) binds directly to the thyroid and stimulates T_4 production. Because T_4 production is independent of TSH production, the usual regulatory mechanisms are ineffective in its control.

1. How is T_4 involved in temperature regulation? _____

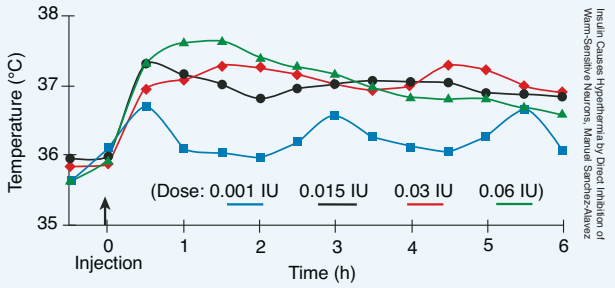
2. Explain how T_4 production is regulated by negative feedback: _____

ISBN: 978-1-98-856655-9

Insulin and thermoregulation

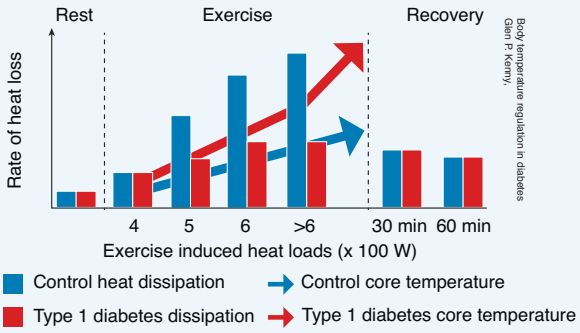
- Insulin is a hormone that is usually associated with regulation of blood glucose, signalling cells to take up glucose from the blood. The cells then use this glucose to produce ATP for use in metabolic processes. However, research has also shown that insulin can directly affect core body temperature.

Body temperature response to insulin in mice



Experiments have shown that, when insulin is injected into the preoptic area of the hypothalamus, there is a rapid, hyperthermic (increased temperature) response, caused by the stimulation of thermogenesis in brown fat. This may help to explain why people with diabetes, whose cells do not produce insulin or do not respond to insulin, often have difficulty with thermoregulation.

Dissipation of heat and core body temperature



Insulin also indirectly affects thermoregulation. People with type 1 diabetes (a lack of insulin production) and type 2 diabetes (insulin resistance) often suffer from thermoregulation problems. In particular, type 2 diabetes results in impaired vasodilation, affecting the ability to dissipate heat. Diabetes is also associated with impaired sweat production, also affecting the ability to dissipate heat.

3. Why do high levels of T_4 not inhibit its production from the thyroid gland in a person with Graves' disease?

4. (a) Would you expect someone with an overactive thyroid gland to feel hot or cold? _____

   (b) Would you expect someone with an underactive thyroid gland to feel hot or cold? _____

5. What was the effect of injecting insulin into the preoptic area of the hypothalamus? _____

6. (a) Describe the relationship between exercise-induced heat loads and core body temperature:

   (b) How does this differ between people with type 1 diabetes and without (control)? _____

7. Why does impaired vasodilation affect thermoregulation in people with type 2 diabetes? _____

ISBN: 978-1-98-856655-9

200 Water Regulation in Plants

Key Idea: Plants maintain homeostasis of water balance by detecting changes in the internal environment and respond by using several mechanisms.

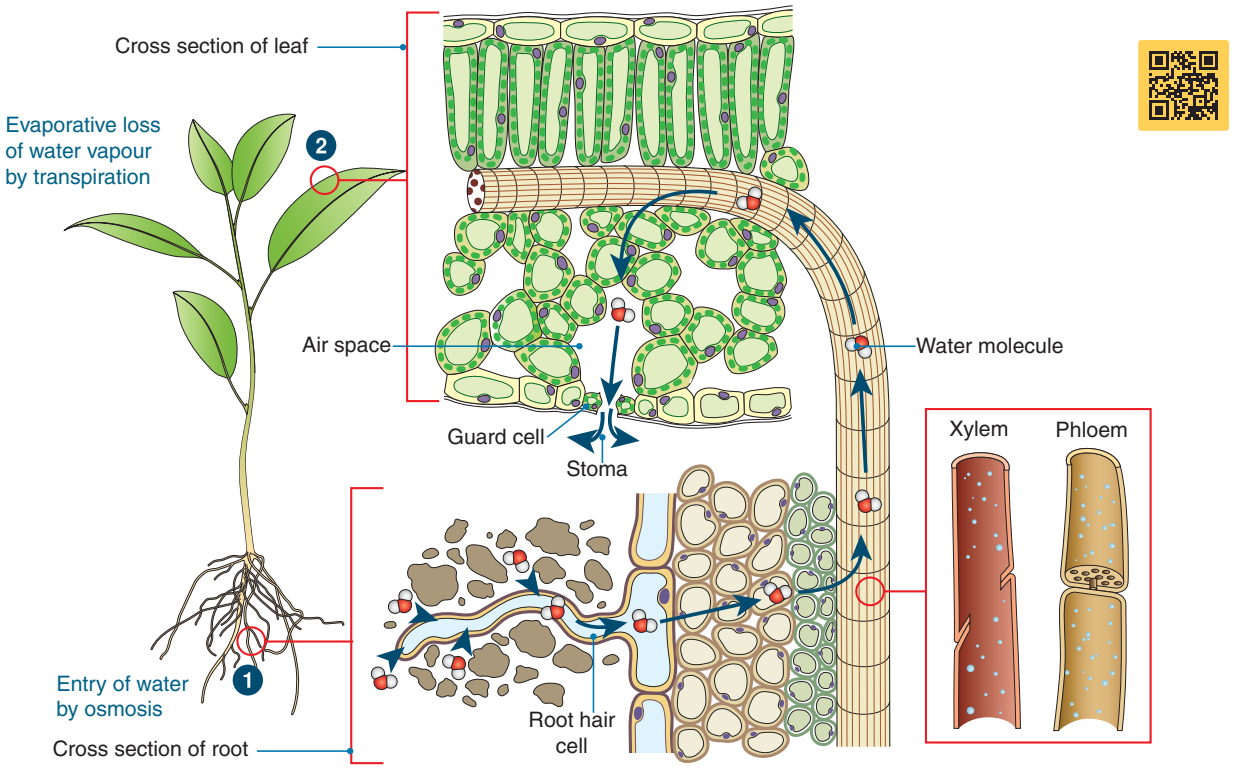
Water is essential for life processes in plants. It is split during photolysis to 'power' the photosynthesis cycle and it ensures turgor pressure in cells. The amount of water, and rate at which it moves through the plant, is balanced by various mechanisms to maintain homeostasis. Water moves through a plant in a one way direction, entering via root hairs by osmosis, transported through the xylem network, and is finally transpired from the stomata. The rate of transpiration determines the volume of water flowing through a plant, and can be controlled by the opening or closing of stomata; this is the key mechanism of plant water regulation. However, the need to close stomata to reduce transpiration competes with the requirement of open stomata for carbon dioxide gas (CO_2) uptake. Transpiration is also used to regulate internal temperature and mineral concentration in the plant.

Movement of water through a plant

Water movement through a plant is facilitated by adaptations of the roots, shoots, and leaves. The control of this water movement to maintain homeostasis occurs in two main structures:

1. Water moving into plants at root hairs → changes osmotic rate by altering dissolved solute concentration, hence water potential.

2. Water moving out of plant from leaf stomata → changes transpiration rate by altering the turgor of stomatal guard cells.



1. Why is maintaining water balance in plants so crucial? _____

_____

_____

_____

_____

2. Explain the significance of the competing processes of photosynthesis and water balance in plants: _____

_____

_____

_____

_____

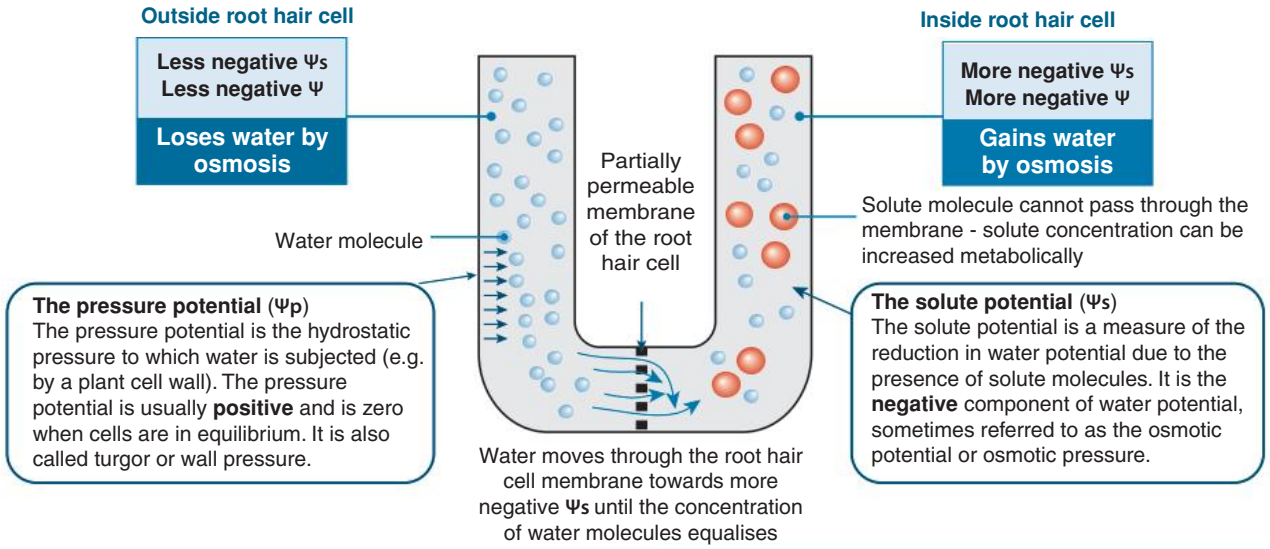
_____

ISBN: 978-1-98-856655-9

Water potential and the control of water intake in root hairs

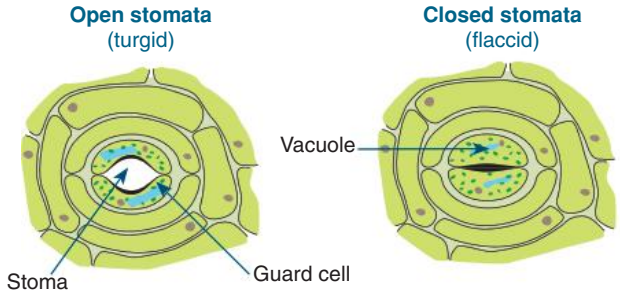
- The water potential (ψ) of a solution inside the root hair cells is derived from combining a value for solute potential (dissolved solute concentration) and the pressure potential (hydrostatic turgor pressure). Water always moves from a region of high ψ to low ψ. Plants are able to maintain homeostasis of internal water balance by metabolically manipulating the concentration of dissolved solutes in the root hair cell, where an increase of solute concentration will lower ψ and increase water uptake via osmosis.

Model of osmotic movement of water across the cell membrane of the root hair



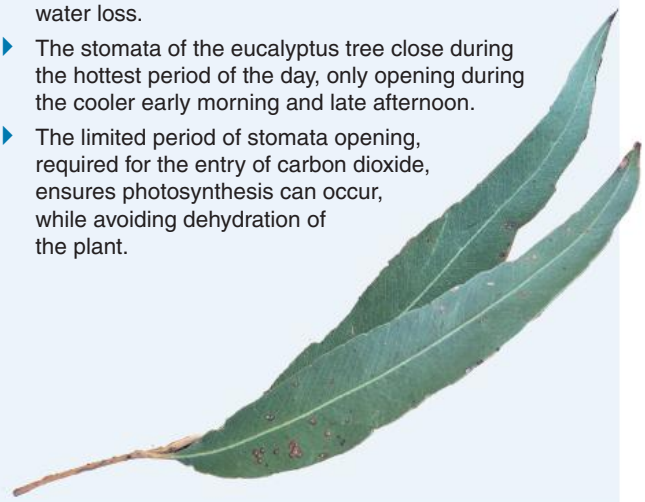
Control of stomata

- Decreased internal plant water levels stimulate the release of a stress hormone, abscisic acid, which then initiates the closure of stomata. Inside the guard cells of the stomata, the water potential (ψ) is increased by opening Ca^+ channels in the membrane, resulting in decreased concentration of solutes. Water then moves out of the guard cells and the flaccid cells close, preventing further transpiration.
- Phototropins inside guard cells register the external stimuli of blue light and, when the plants have adequate water balance, guard cells become turgid due to a lowering of ψ. This causes the cells to bend and open, allowing transpiration to commence once more.



Stomatal control in eucalyptus

- Control of water balance is particularly crucial in dry environments, when stomata remaining open during the entire day would result in excessive water loss.
- The stomata of the eucalyptus tree close during the hottest period of the day, only opening during the cooler early morning and late afternoon.
- The limited period of stomata opening, required for the entry of carbon dioxide, ensures photosynthesis can occur, while avoiding dehydration of the plant.



3. Scientific models can be used to help understand complex ideas.
   (a) What are some strengths of the 'osmotic movement in root hairs' model? _____

   _____

   _____

   (b) What are some weaknesses of the 'osmotic movement in root hairs' model? _____

   _____

   _____

4. What role does the hormone, abscisic acid, play in water balance? _____

   _____

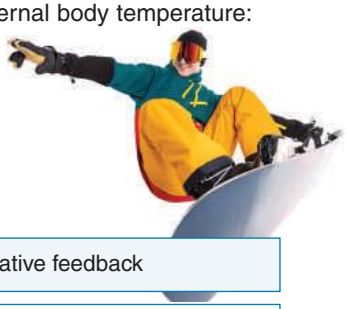
   _____

ISBN: 978-1-98-856655-9

201 Chapter Review: Did You Get It?

1. How does the behaviour of a negative feedback system maintain homeostasis? _____

2. Complete and add detail to the following thermoregulation negative feedback loop for **low** internal body temperature:



3. Explain **how** a mechanism is able to effectively counter each stress, to maintain homeostasis of blood glucose:

   (a) Blood glucose too high: _____

   (b) Blood glucose too low: _____

4. Contrast the mode of action of the nervous and endocrine systems in maintaining homeostasis: _____

5. **Search online** and elaborate on **mountain pygmy possum** adaptations that allow it to maintain thermal homeostasis:

Small ears - structural	Winter torpor - physiological	Nocturnal - behavioural

6. Explain the processes in plants that maintain water balance, as well as controlling transpiration rate from the stomata:

ISBN: 978-1-98-856655-9

CHAPTER 14

Cause and Response

Activity number

Key terms

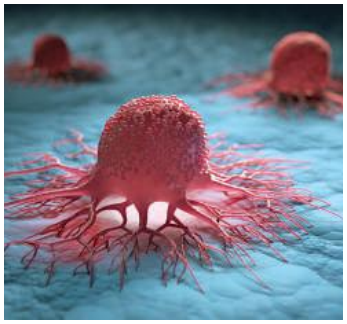
anaemia
asbestos
asbestosis
carcinogens
chronic
COPD
Cri du chat syndrome
Edwards' syndrome
goitre
incidence
mesothelioma
morbidity
mortality
non-infectious disease
Patau syndrome
period prevalence
phenylketonuria (PKU)
point prevalence
prevalence
rickets
risk-factors
Tay-Sachs disease

Inquiry question: *Do non-infectious diseases cause more deaths than infectious diseases?*

Causes and effects of non-infectious diseases

Key skills and knowledge

- ☐ 1 Define a non-infectious disease. Identify some examples of non-infectious diseases linked to provided risk factors. **202**
- ☐ 2 Understand the four main categories of non-infectious diseases: genetic disorders; diseases caused by environmental exposure; nutritional diseases; and cancer. Match non-infectious diseases to causes and effects. **203**
- ☐ 3 Relate a variety of diseases to different types of environmental exposure. Contrast the duration and severity of genetic disorders/diseases and diseases caused by environmental exposure. Link causes and effects in a case study of environmental exposure diseases. **203**
- ☐ 4 Review a range of nutritional disease case studies, linking cause and effect. Interpret data to gain an understanding of multiple causes of cancer. **203**
- ☐ 5 Work in small groups or pairs to search online causes and effects of a provided disease. Collaborate to establish common causes and effects of two related diseases. Collaborate to identify unique causes and effects of two related diseases. **203**

Incidence, prevalence, and mortality data

Key skills and knowledge

- ☐ 6 Define the terms incidence, prevalence, and mortality in the context of non-infectious diseases and disorders. **204**
- ☐ 7 Calculate the incidence rate of lung cancer in Australian men and women from secondary data. **204**
- ☐ 8 Explain the usefulness of incidence and prevalence data to inform public health. **204**
- ☐ 9 Interpret secondary data representing the impact of different socio-economic aspects on the prevalence of childhood obesity in Australia. Use a fishbone chart to analyse cause and effect factors in childhood obesity. **204**
- ☐ 10 Understand how mortality rate data can inform health decisions. **204**
- ☐ 11 Link terms and definitions in the context of non-infectious disease data representation. **204**

202 What is a Non-infectious Disease?

Key Idea: Non-infectious diseases, with the exception of inherited conditions, cannot be transmitted from person to person, and are often caused by genetic or lifestyle factors.

Non-infectious, or non-communicable, diseases (NCD), cause more human deaths than infectious diseases. A variety of risk factors increase the likelihood of someone developing a non-infectious disease. These include poor lifestyle choices, environmental exposure, and inherited mutations. The effects of non-infectious disease are often permanent and/or chronic (long term), and slow to progress.

Non-infectious disease

Non-infectious diseases can be grouped into four main categories based on their underlying causes:

Genetic diseases/disorders

Genetic diseases are often rare and are caused by mutations (DNA errors) carried by parents and passed to offspring. They may also occur spontaneously during the development of the sperm or egg. Effects can range from minor impacts to fatal conditions. Inherited diseases include Huntington's disease, cystic fibrosis, and Down Syndrome (right).

Diseases caused by environmental exposure

These diseases are caused by external factors, for example excessive UV radiation exposure leading to skin cancer, or poisoning from toxic heavy metal pollution such as mercury or lead. These diseases can also be created by poor lifestyle choices, including excessive alcohol consumption or smoking.

Nutritional diseases

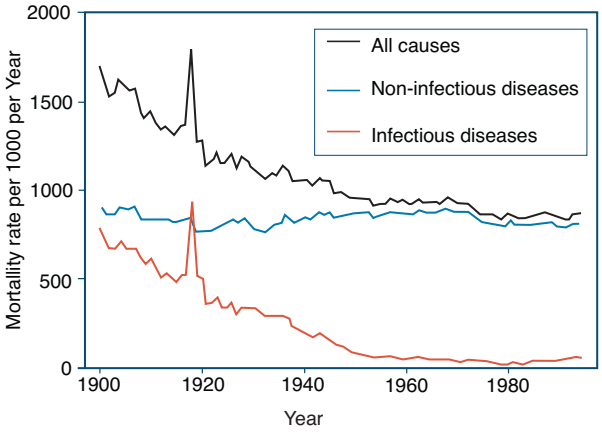
These diseases can develop from either a deficiency or excess of one or more components in the diet. Lack of vitamins, minerals and trace elements in the diet can cause a wide range of debilitating conditions, e.g. anaemia (lack of iron). Diets of excess carbohydrates or fats can lead to obesity, type 2 diabetes, and cardiovascular disease.

Cancer

Cancer is caused by mutations in the genes that keep cell growth and division in check. Cancer causes cells to grow and divide continuously, causing tumours and affecting organ function. Exposure to carcinogens (cancer causing agents) such as tobacco smoke, toxic chemicals, ionising radiation, and some pathogens increases the risk of cancer. Some cancers can be caused by inherited genes.

Mortality rates for non-infectious vs infectious disease

Human mortality rates associated with infectious diseases and non-infectious diseases in the USA during the 20th century



Armstrong et al., 1999. See credit for full reference

1. Complete the table below by linking the risk factor to some possible non-infectious diseases. Some risk factors may influence the likelihood of developing more than one disease. You may have to carry out some research.

Risk factor	Non-infectious diseases caused
Tobacco smoking	(a)
Excessive UV sunlight exposure	(b)
Lack of iron in food	(c)
Genetic mutations occurring during meiosis	(d)
Excessive consumption of food high in sugar	(e)
Heavy metal poisoning i.e. lead or mercury	(f)
Excessive alcohol consumption	(g)

ISBN: 978-1-98-856655-9

203 Causes and Effects of Non-infectious Disease

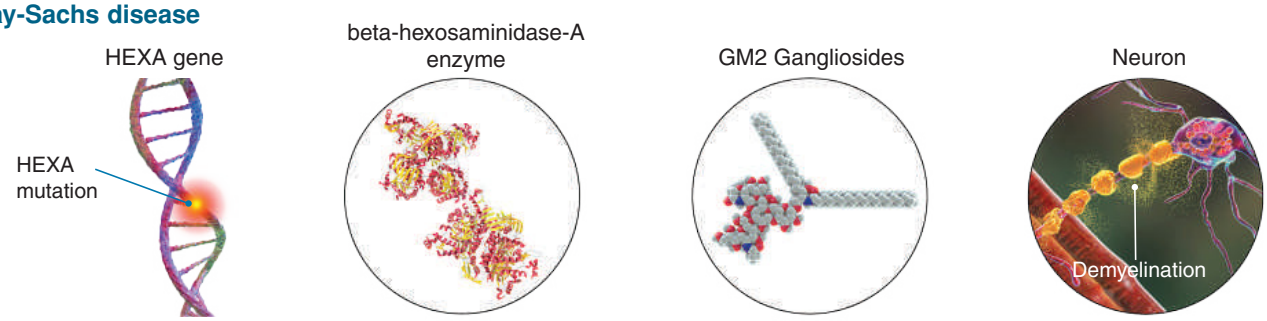
Key Idea: Non-infectious diseases have many causes, including inherited genetic mutations, harmful environmental exposure, and nutritional imbalance. They have different effects on the body.

Although we can classify non-infectious diseases into four main categories, there is substantial cross-over. For example, the risk factors for various cancers can be due to inherited faulty genes, nutrition, or harmful environmental exposure. Any particular disorder can present a range of symptoms and vary in severity due to a number of factors. Categorising disease is useful for investigating patterns and trends across a very broad group of non-infectious diseases.

Diseases caused by genetic abnormalities

- Genetic mutation, covered in detail in Module 6, is the most common cause in this category of non-infectious diseases.
- **Chromosome mutations,** responsible for many inherited diseases, create numerical or structural changes to the chromosomes and are passed on via germ-line mutations in the gametes. Many of these diseases impact multiple areas of the body from birth, depending on which genes are affected, and include Down syndrome and Turner syndrome.
- **Point mutations** occur at DNA level when one or more base pairs have been deleted, added, or substituted, creating a defect in a gene which is passed to the next generation via gametes. This type of mutation is typically recessive, but when the disease is present in homozygous individuals it can have major implications for body growth and development, and even result in premature death. Examples include cystic fibrosis, Huntington's disease, and Tay-Sachs disease (shown in more detail below).

Tay-Sachs disease



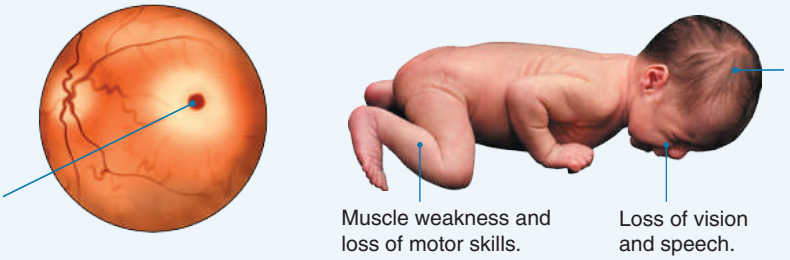
Tay-Sachs is an autosomal recessive disease caused by a mutation in the HEXA gene on chromosome 15. This prevents the formation of a vital enzyme, beta-hexosaminidase-A.

In the absence of beta-hexosaminidase-A, fatty substances known as GM2 gangliosides build up to toxic levels in the brain and spinal cord.

Symptoms

The most severe variant of Tay-Sachs disease is the infantile form, where symptoms begin to appear from 3-6 months, and progressively get worse until death within a few years.

Red spots develop in the retina and interfere with vision (right).



1. Describe the difference in the origin of chromosome mutations and point mutations: _____

_____

_____

2. **Match** the causes and/or effects to the correct genetic diseases by doing an online search.
Diseases to select from: *phenylketonuria (PKU), Patau syndrome, Cri du chat syndrome, Edwards' syndrome.*

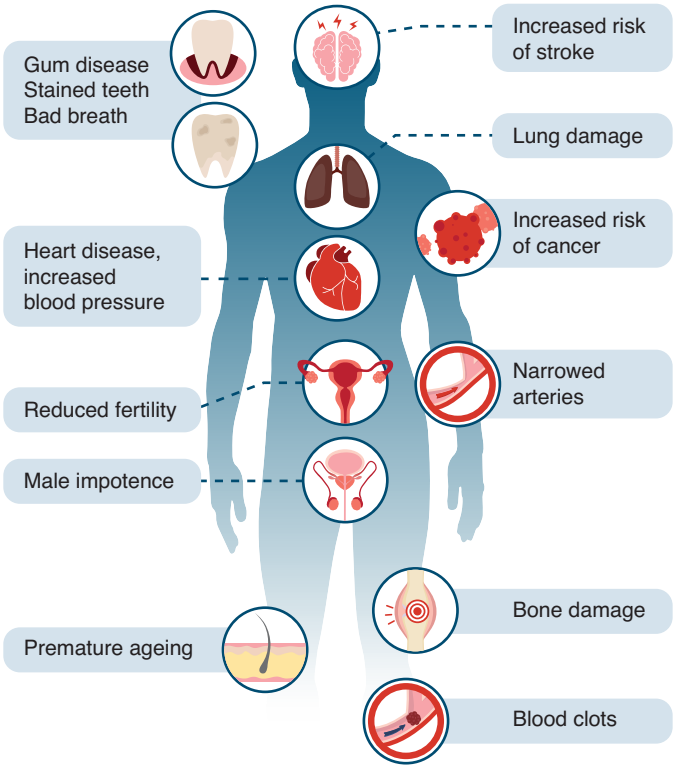
Genetic disease	Cause or effect of disease
(a)	Trisomy mutation of a chromosome. 1 in 10 will experience mosaic trisomy, exhibiting a less severe form.
(b)	Trisomy mutation of a chromosome. Around 1 in 20 will experience mosaic trisomy.
(c)	Mutation in a gene that produces an enzyme to process a particular amino acid. Recessive trait.
(d)	Deletion mutation in a chromosome impacting multiple genes. Usually the result of a random event.

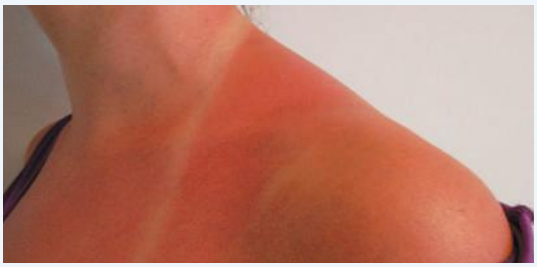
Genetic disease	Cause or effect of disease
(e)	Few babies survive severe form. Milder forms will cause heart conditions, feeding difficulties, and low set ears.
(f)	Progressive disease in newborns seen after a few months, with neurological problems, and microcephaly.
(g)	High pitched cry in infants, microcephaly, wide-set eyes, and intellectual disability.
(h)	Restricted growth in uterus, low birth weight, usually with severe heart defects. May have forebrain failure.

ISBN: 978-1-98-856655-9

Diseases caused by environmental exposure

- Lifestyle choices can influence the risk of developing many of the diseases in this category. However, some diseases are caused by exposure to toxic substances, poor air quality, and heavy metals. The risks may go undetected until symptoms start appearing. A notable feature of many of these diseases is their widespread harmful effects on multiple organs.
- Environmental exposure to tobacco smoke is a well-recognised risk factor for acute and chronic respiratory illness. Tobacco contains many toxic and carcinogenic substances that enter the body when smoke is inhaled. Carcinogens cause damage to DNA or disrupt cellular processes, leading to the formation of cancerous tumours.
- Exposure to tobacco smoke is the most common cause of lung cancer, and also causes mouth and throat cancer. Smoking also causes a range of other diseases and chronic illnesses (below).





Exposure to electromagnetic radiation
Exposure to UV radiation damages skin. Long or intense exposure can cause significant burns, so that skin is lost (peeling). Repeated, long-term damage increases the risk of various, often fatal, cancers.

Exposure to industrial waste
Heavy metals (e.g. lead or mercury) present in older paints, water pipes, or industrial residue can cause lasting damage to neurological systems and multiple organs. Excessive exposure, without treatment may eventually lead to death.

Excess consumption of alcohol
Alcohol is broken down in the body into acetaldehyde, a harmful substance that is processed by the liver. Long term, excessive alcohol consumption can lead to numerous diseases, including cirrhosis of the liver (above), heart disease, and cancers.

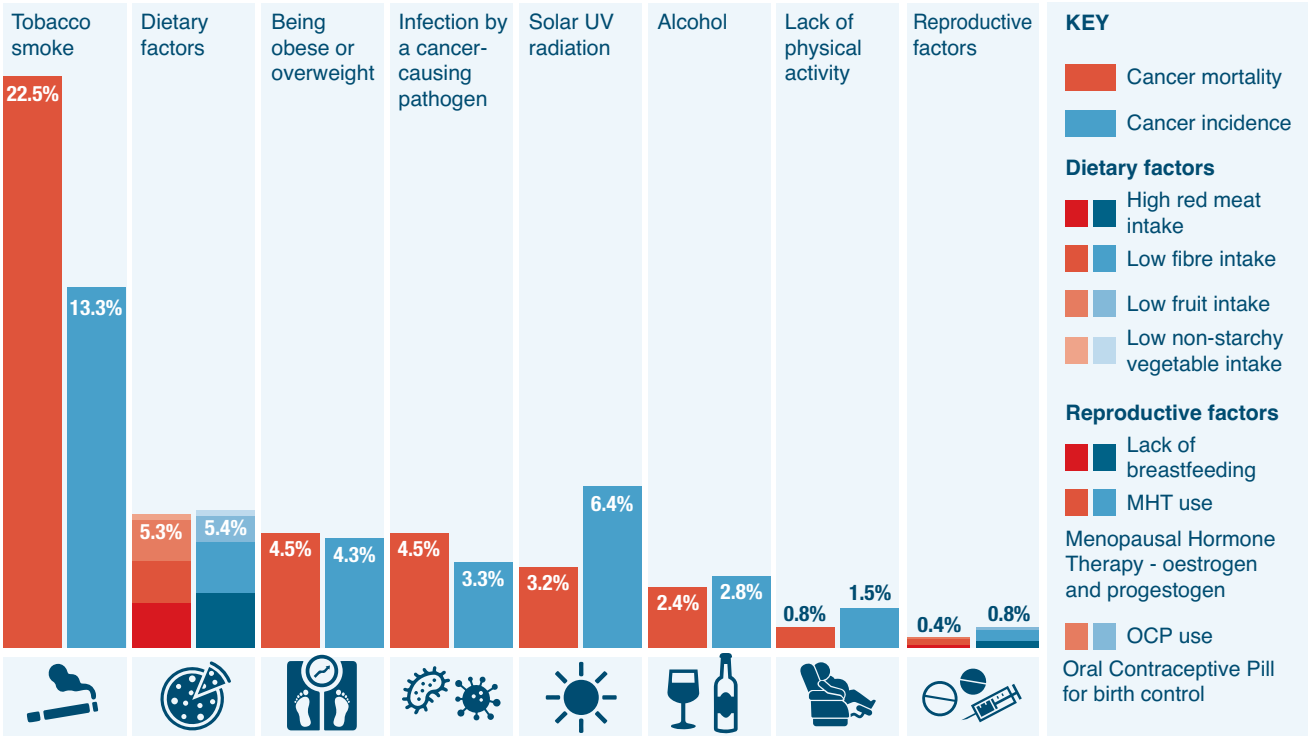
3. How does the duration and severity of diseases caused by environmental exposure differ from genetic diseases?

4. Immediately following bush fires in NSW, hospital emergency admittances for asthma and chronic obstructive pulmonary disease rose 23% and 12%, respectively. What is the likely causal link between these events?

ISBN: 978-1-98-856655-9

Cancer

- This category of non-heritable disease is identified by uncontrolled cell division due to regulatory checkpoint failure in the cell cycle. Continued growth of these abnormal cells leads to tumour formation and can spread to other parts of the body, leading to death if untreated. The specific **causes of cancer** (summary graph below) are often linked to the tumour location and type of cancer that forms.

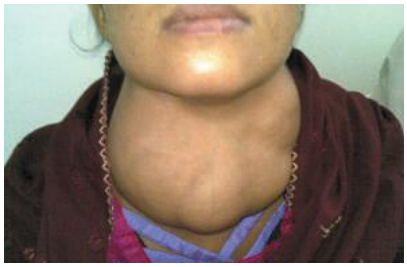


Data source: Wilson et al. (2018). How many cancer cases and deaths are potentially preventable? Estimates for Australia in 2013. Int. J. Cancer: 142 (4), 691-701.

Nutritional diseases

- Historically, nutritional disorders were due to a general lack of access to adequate food. For example, sailors at sea for long periods of time, without food containing vitamin C, contracted scurvy. Now, personal food choices such as vegan diets; iodine-free sea salt; or consumption of excess food, leading to obesity, can increase the risk of developing a nutritional disease.

Iron deficiency: anaemia
Iron is the oxygen-carrying component of haemoglobin in red blood cells and low iron levels lead to anaemia. Lack of oxygen to cells leads to pale skin and fatigue. Red meats are iron-rich, so vegetarian diets need to include an alternative source of iron. Menstruation causes women to be in the greatest risk group for this disease.



Iodine deficiency: goitre
Dietary iodine deficiency leads to swelling of the thyroid gland at the base of the neck, called goitre. Most table salt and bread in Australia has been iodised as a general health measure to ensure adequate intake. Diseases of the thyroid can affect many aspects of a body's metabolism. Excess iodine can also cause a form of goitre.

Vitamin D deficiency: rickets
Calcium and phosphorus are required for bone growth and maintenance. Absorption of these minerals is helped by vitamin D, which is found in some foods and converted from cholesterol when skin is exposed to sunlight. Young children with rickets (above), show severe deformities in their legs compared to the normally-developed child in the centre.

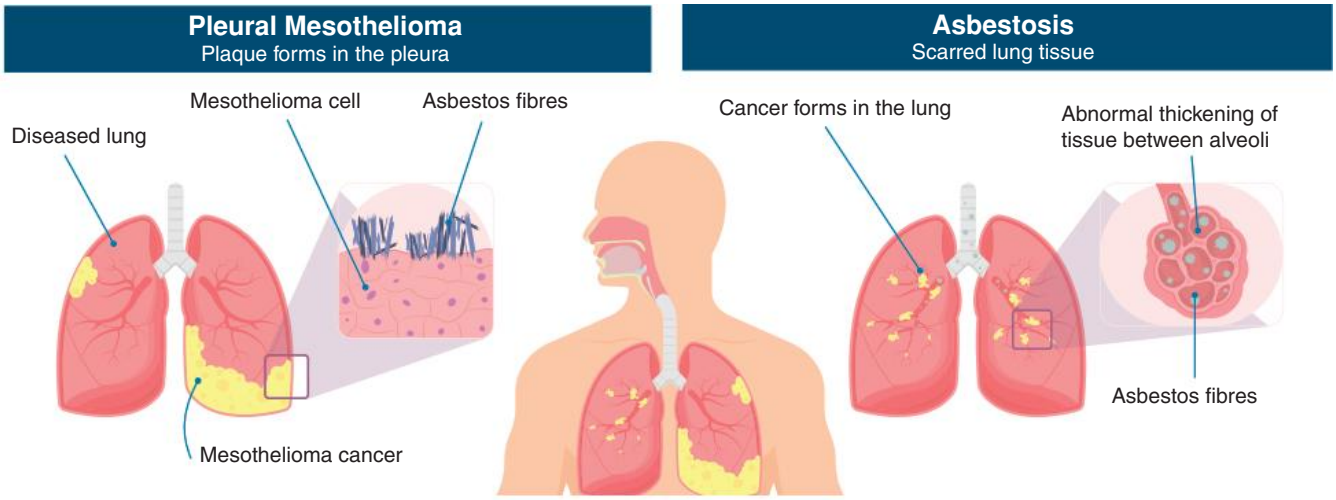
5. Goitre incidence has reduced significantly in Australia. Iodine was added to salt in 1924 and the addition of iodised salt to bread was made compulsory in 2009. However, iodine levels have been reducing in some population groups once more. What reasons might there be for this trend?

6. What are the three most common causes of cancer mortality? _____

ISBN: 978-1-98-856655-9

Mesothelioma vs Asbestosis

- Asbestos is a naturally occurring substance and is used as building insulation and ceiling coatings in older buildings. Asbestos is a carcinogen and exposure to it, especially during removal, can lead to cancer as well as severe lung disease.
- **Mesothelioma** is a rare, mostly fatal, type of cancer that is almost exclusively acquired from asbestos exposure. However, more typical types of lung cancer can also develop.
- A second disease linked to asbestos exposure, **Asbestosis,** has higher survivability rates but requires long term treatment to mediate the effects.



7. (a) Work in pairs or small groups to search online causes and effects of either mesothelioma **or** asbestosis disease. Record your notes on spare paper.
   (b) Join pairs/groups together and create an agreed list of **common** causes and effects between the two diseases, as well as those that are **unique** to each disease. Record key points in the table below.

Pleural mesothelioma Cancer causing abnormal and malignant growth of the pleural layer of the lungs.		**Asbestosis** Chronic lung disease caused by asbestos fibres that can cause lung tissue scarring.
(c) **Unique causes** of mesothelioma	(d) **Common causes**	(e) **Unique causes** of asbestosis
(f) **Unique effects** of mesothelioma	(g) **Common effects**	(h) **Unique effects** of asbestosis

ISBN: 978-1-98-856655-9

204 Incidence, Prevalence, and Mortality of Disease

Key Idea: The incidence, prevalence, and mortality of non-infectious diseases is a useful way of comparing data across different and diverse groups.

Non-infectious disease data can be from both primary and secondary sources. Recall that, for any given disease, **incidence** is defined as the rate of new cases occurring in a given population or group over a time period. Incidence is focussed on disease growth or reduction, and **prevalence** is a measure of the proportion of the population with a disease at any given time. It provides information about the disease burden that a studied group or population carries. Both incidence and prevalence data can be expressed as a proportion or percentage. **Mortality** is used to indicate how many in a particular population have died of a specified disease. Mortality rate is often expressed in number per 100,000 or 1,000,000 individuals.

Reported lung cancer disease in men and women in Australia (2005-2019)

Year	Males with lung cancer	Females with lung cancer	Estimated population female	Estimated population female	Incidence per 10,000 male	Incidence per 10,000 female
2005	5878	3540	9,952,665	10,093,338		
2006	6148	3652	10,087,382	10,224,161		
2007	6118	3874	10,248,923	10,378,624		
2008	6198	4051	10,449,776	10,566,345		
2009	6144	4296	10,689,738	10,785,887		
2010	6327	4211	10,886,022	10,979,601		
2011	6406	4233	11,034,979	11,137,490		
2012	6568	4685	11,206,252	11,315,945		
2013	6675	4653	11,408,788	11,519,235		
2014	6601	4887	11,582,448	11,715,329		
2015	6779	5009	11,744,498	11,895,833		
2016	6841	5088	11,904,308	12,080,273		
2017	6952	5256	12,098,224	12,291,460		
2018	7088	5458	12,285,452	12,487,898		
2019	7184	5633	12,482,498	12,688,793		

data sourced from https://ncci.canceraustralia.gov.au/ and https://www.abs.gov.au/statistics

Incidence

To calculate the incidence of any given disease, in any given time period, two types of data are required:

1. The total number of **new cases** of the disease in a specific population appearing during the specified time period.

2. The **size of the population** at the start of the specified time period.

The incidence proportion can then be calculated:

$$\text{Incidence} = \frac{\text{Number new cases}}{\text{Size of population}}$$

To more clearly compare incidence per time period, usually a year, when the population size is changing, it is useful to convert incidence to a set number of cases per size.

For example: per 10,000

$$\text{Incidence per } \mathbf{10{,}000} = \frac{\text{Incidence}}{100} \times \mathbf{10{,}000}$$

1. Complete the table by calculating the male and female incidence of lung cancer per 1,000,000 (million), per year, in Australia from 2005-2019 (left).

2. How might incidence data of a specific disease be useful to a public health unit or ministry?

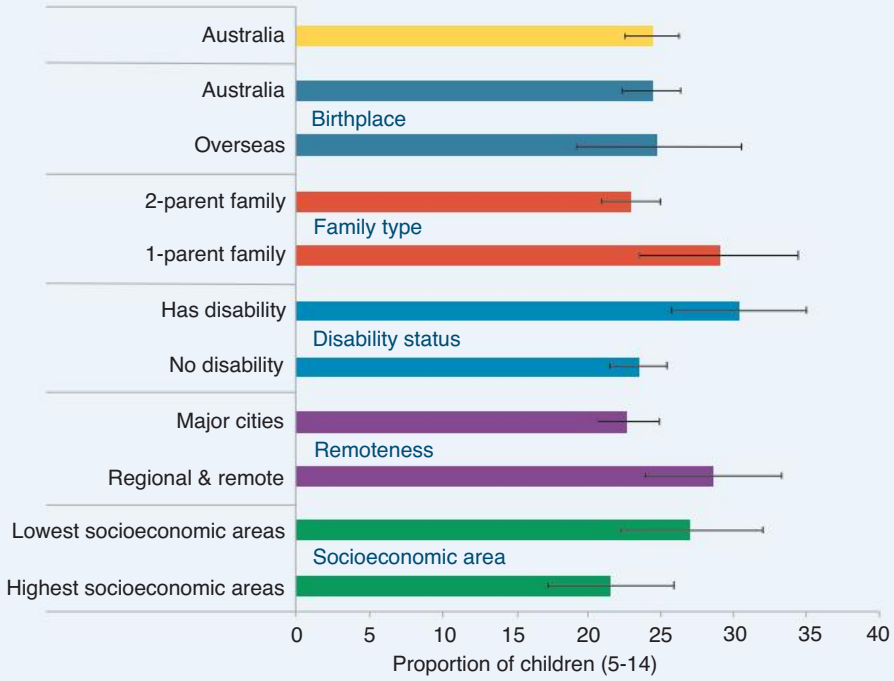
3. What extra data would be useful to show the burden of a specific **chronic**, but usually not fatal, disease on a healthcare system, and why does the incidence data not show this?

ISBN: 978-1-98-856655-9

Prevalence

- Prevalence data can be presented in two forms: **point prevalence**, which provides the proportion of a population diagnosed with a specific disease at one particular point in time, and **period prevalence**, where the proportion of a population with the specific disease is related to a period, for example, over a year.
- Once a person dies or is cured of the specific disease, they are removed from the prevalence data set. Although an increase in prevalence may correlate to an increase in incidence, this is not always the case. A new medicine that prolongs the life of individuals with chronic disease will also see an increase in prevalence, even if the incidence rate remains consistent or falls.

Proportion (%) of children aged 5-14 who were overweight or obese, by population groups, 2017-2018

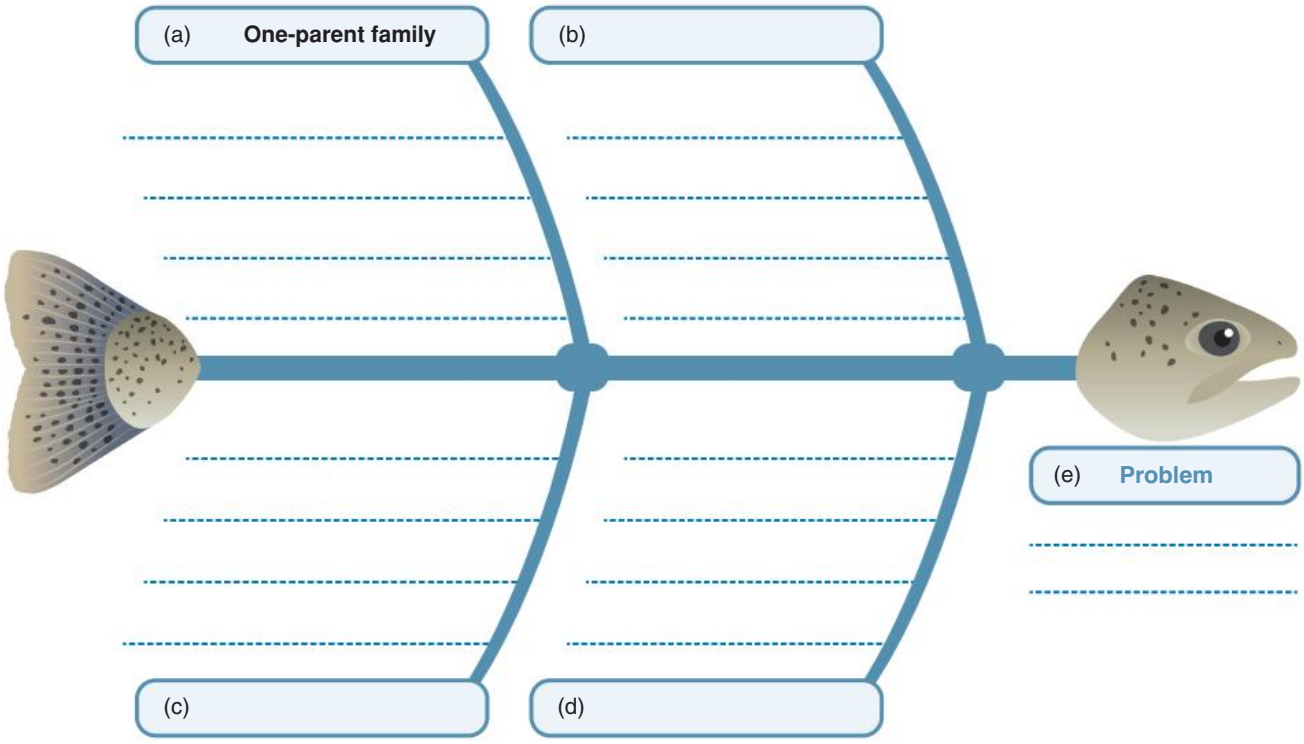


Obesity is an overweight condition measured by BMI (body mass index) and can be seen as an excessive amount of body fat. Obesity leads to poorer health outcomes, decreased mobility, and an increase in the risk of adult illness and mortality.

Obesity is classified as a non-infectious nutritional disease caused by poor and excessive food intake, especially of fats and carbohydrates.

Data soure: ABS 2019a. Microdata: National Health Survey, 2017–18. ABS cat. no. 4324.0.55.001. Canberra: ABS. Customised data report.

4. From the data above, **four factors** are shown as contributing more significantly to a higher prevalence of childhood obesity. A **fishbone chart** allows classification of multiple causes that contribute to an outcome. Write the problem; list each factor as a heading; and conduct an online search to find information for factors which might increase the prevalence of childhood obesity, listing these underneath each factor. One heading has been completed for you. Discuss your findings in a group or as a class.



* Students must remember that correlation does not always equal causation

ISBN: 978-1-98-856655-9

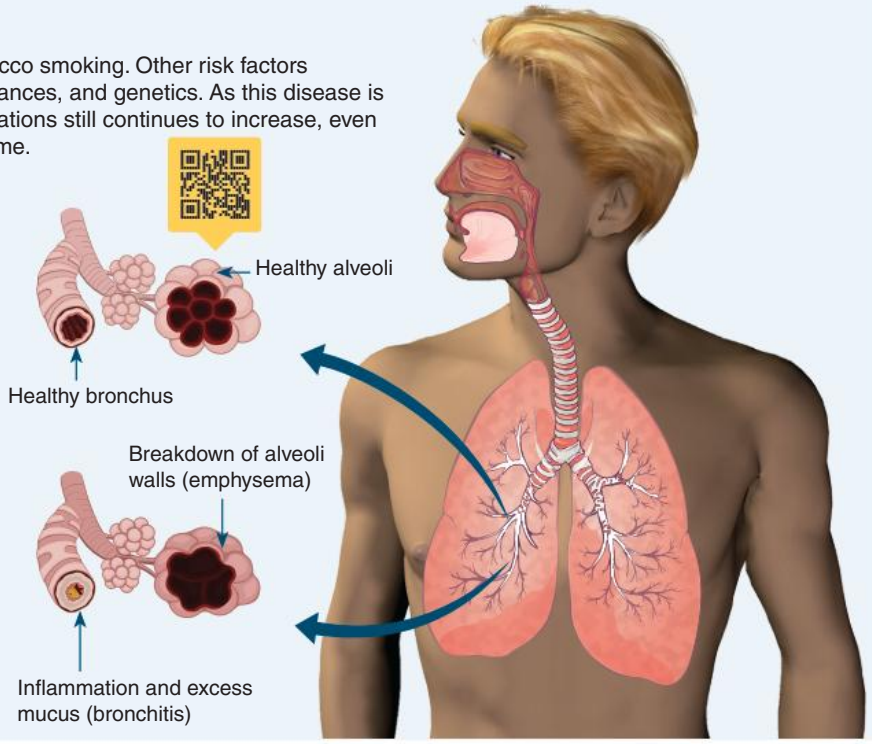
Chronic Obstructive Pulmonary Disease (COPD)

COPD is a type of chronic lung disease that causes persistent respiratory symptoms, including difficulty in breathing. Major contributors to COPD are emphysema (the formation of air-filled spaces in the lungs) and chronic bronchitis (excess mucus in the lungs).

The most common cause of COPD is tobacco smoking. Other risk factors include pollution, exposure to irritant substances, and genetics. As this disease is progressive, the prevalence in many populations still continues to increase, even though tobacco use has decreased over time.

In the graph below, COPD mortality is expressed as the rate of deaths per 100,000 in the specific population. Three features of this data make it useful for further analysis and to inform health organisations:

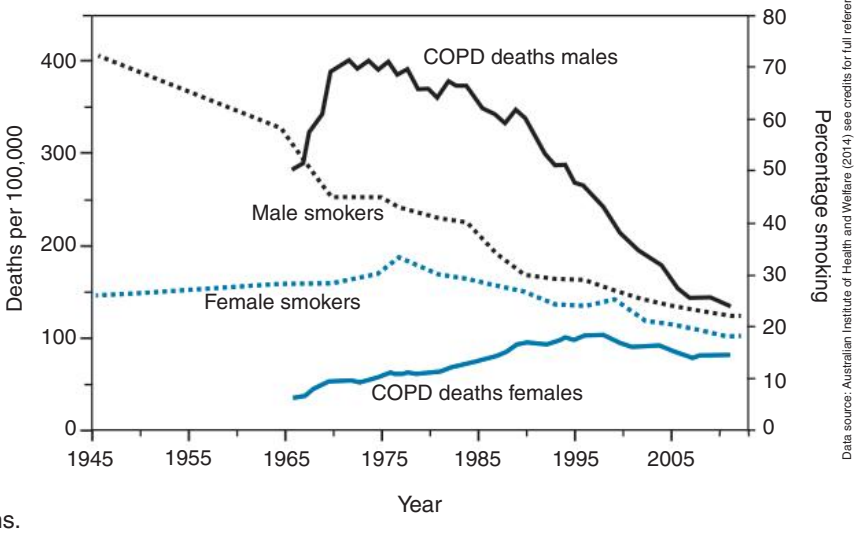
- Mortality data is shown over a lengthy period to reveal trends, if they are present.
- The population is split into groups (male and female) to compare mortality between them.
- Prevalence of a possible cause or risk factor for the disease can be overlaid on the graph to reveal correlations in the data.



COPD mortality

- **Mortality**, or death rate, measures the number dying per unit time. **Morbidity** is a measure of those afflicted by a disease.
- Here, we are looking at mortality due to a pre-existing non-infectious disease.
- Mortality data can be represented in various ways:
- **Deaths per million/thousand** indicate the total impact on the health of a population.
- **Mortality-to-case data**, or case fatality rate, informs on the proportion of individuals who die from the same disease within a specific period. It is a measure of disease severity.
- Mortality data can be used to make predictions about future disease outcomes in a population.

Mortality due to COPD (Australia) in people 55+, 1964-2011 and smoking rates, 1945-2011, by sex



Data source: Australian Institute of Health and Welfare (2014) see credits for full reference

5. Match the terms in the box to their definitions.

incidence	prevalence	mortality	morbidity	point prevalence	period prevalence	mortality rate	survival rate

Term	Definition
(a) _____	The rates of a disease in a specific population.
(b) _____	The number of deaths per disease, per population, per period.
(c) _____	The total number of disease cases, or disease burden, in a specific population.
(d) _____	The proportion of people who have not died from a specific disease in a specific, relevant, time period.
(e) _____	The total burden of a specified disease during a specified time period.
(f) _____	The number of deaths scaled to a population, i.e. per 1,000,000 or 100,000
(g) _____	The number of new cases of disease in a specified period.
(h) _____	The total burden of a specified disease at a given time.

ISBN: 978-1-98-856655-9

205 Chapter Review: Did You Get It?

1. Certain types of cancer, such as breast, prostate, and ovarian cancer, have been linked to an inherited gene. Why are they unlikely to be classified as genetic disorders in the same way that Down syndrome or cystic fibrosis are classified?

2. Incidence and prevalence data are useful metrics for public and population health, but both sets are usually required for a clearer analysis of any trends. If a PHN (Primary Health Network) experienced a 15% increase in **prevalence** of pancreatic cancer in their population in the past year:

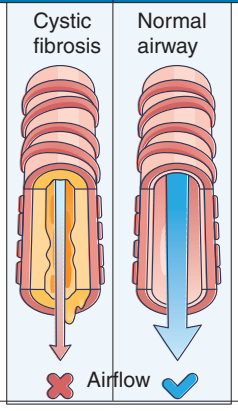
   (a) What are some possible reasons for this rise?

   (b) How might **incidence** data help to indicate whether or not this was a concern?

3. Indicate the effects on disease incidence, prevalence, and mortality over the short and long term, if different:

Situation	Effect on incidence	Effect on prevalence	Effect on mortality
A successful new treatment medicine is released to extend the life of stage 4 breast cancer patients.	(a)		
A new vaccination programme is established in an area to decrease the risk of developing liver cancer.	(b)		
A new programme promoting healthy eating and exercise is successfully introduced into local primary schools to combat childhood obesity.	(c)		
A local industry, that was accidently releasing mercury into a local waterway used as a source of drinking water, has been closed.	(d)		

4. The current number of people with cystic fibrosis in Australia is approximately 3,500. Most newborn babies are screened at birth for this non-infectious disease. Search online for the **causes** and **effects** of this disease:

Causes		Effects
(a)		(b)

ISBN: 978-1-98-856655-9

CHAPTER
15

Epidemiology

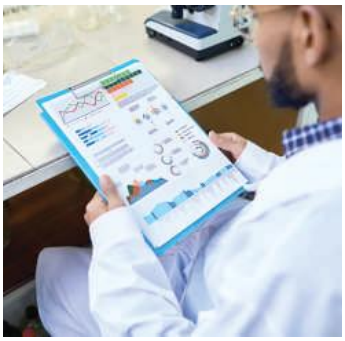
Activity number

Key terms
agent
analytical study
cardiovascular disease
cell therapy
comorbidity
control
descriptive study
epidemiology
external validity
gene therapy
internal validity
interventional study
longitudinal study
observational study
type 2 diabetes
validity

Inquiry question: *Why are epidemiological studies used?*

Pattern analysis of non-infectious diseases

Key skills and knowledge



Current and future disease treatment

Key skills and knowledge

Epidemiological studies

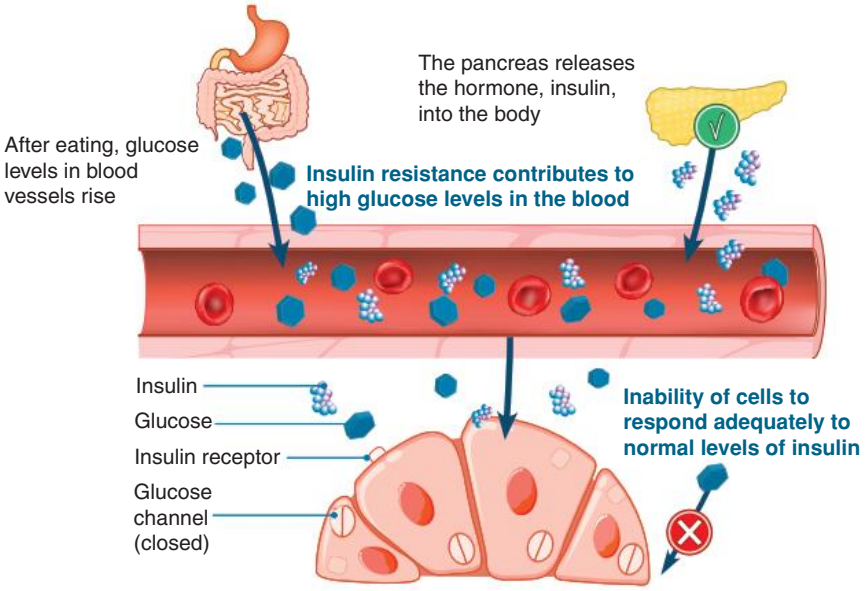
Key skills and knowledge

206 Analysing Patterns of Non-infectious Disease

Key Idea: Analysis of non-infectious disease data can provide a clearer understanding of disease patterns and risk factors, to make treatment and control more effective.

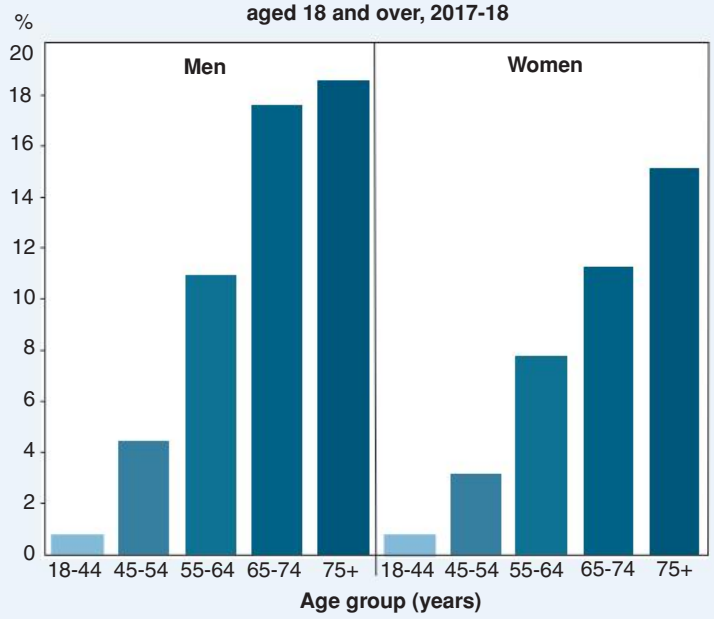
Non-infectious diseases kill over 40 million people a year, according to the World Health Organisation. Over 80% of deaths are caused by cardiovascular disease, cancer, respiratory disease, and diabetes. The greatest risk factors for the majority of non-infectious diseases are tobacco use, excessive alcohol use, physical inactivity, and excessive/deficient nutrition. Data analysis can be used to predict patterns of concern, and then help health organisations implement control and treatment measures, and assess their effects. Data analysis can help in the development of disease awareness and prevention campaigns for the public.

Type 2 diabetes



- Type 2 diabetes, sometimes called adult onset diabetes, is the most common form of diabetes mellitus. This disease is a significant burden on Australian healthcare. Around 5% of those aged 18 and over in the population were diagnosed as having this disease in the 2017-18 period.
- This disease differs from type 1 diabetes in that the main risk factors are lifestyle/nutritional choices rather than genetics.
- Risk factors for developing type 2 diabetes include: consumption of excessive carbohydrate (sugars) and fat leading to obesity, a sedentary lifestyle, smoking, and heavy drinking.

Prevalence of self-reported type 2 diabetes, among persons aged 18 and over, 2017-18



Source: AIHW analysis of ABS 2019a http://www.aihw.gov.au/

Different ways of presenting non-infectious disease data can be useful when looking for meaningful patterns. For example, although we are informed that over 5% of the Australian population have developed type 2 diabetes, more detailed data is required to enable patterns to be seen.

- Breaking population groups into **age brackets** can reveal any patterns where disease prevalence is a significant factor. For example, in type 2 diabetes the prevalence in the 55+ age group is around 10 times higher than under 44. This information can assist with targeted prevention and treatment.
- Breaking population groups into men and women can reveal patterns that enable specific types of prevention and treatment to be better targeted and more effective. For example, although the overall prevalence of type 2 diabetes increases with age for both men and women, the prevalence of type 2 diabetes after the age of 45 becomes higher in men, in successive age groups.

1. Describe why the pattern of prevalence in the graph above might show an increase as the population ages, linked to:

   (a) Incidence: _____

   (b) Mortality: _____

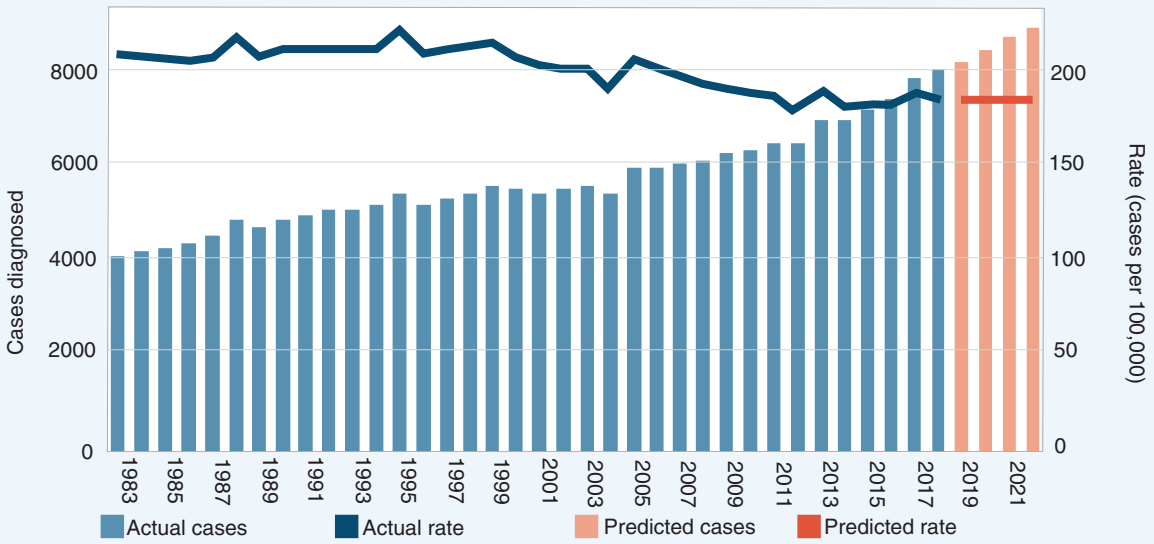
2. What other grouping types might be useful to show patterns in this data? _____

ISBN: 978-1-98-856655-9

Nicotine vaping, tobacco smoking and lung cancer

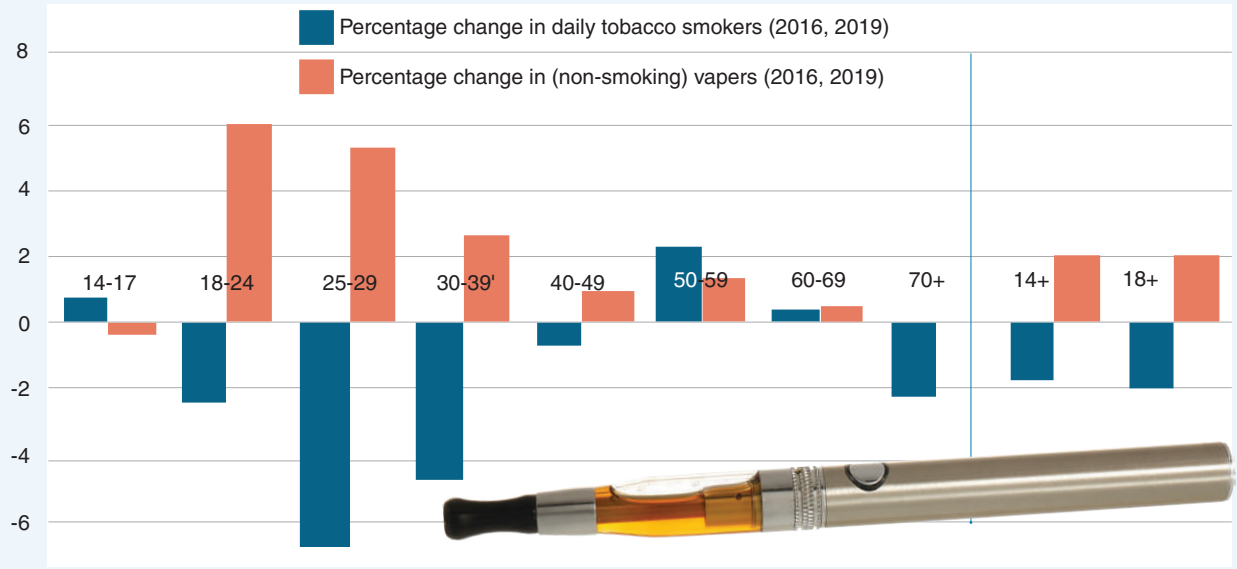
Research has demonstrated a conclusive link between tobacco smoking and the incidence of lung cancer. Nicotine 'vaping', through an electronic device, is a new phenomenon and Australian research links its uptake to a decrease in smoking. Although 'vaping' is often promoted as a safe alternative to tobacco smoking, developing evidence is showing a multitude of possible negative health impacts, including cardiovascular and lung disease. Long-term health impacts are still unknown, which is concerning.

Lung cancer incidence, Australian population 60-79 years



Source: AIHW http://www.aihw.gov.au/

Change in percentage of daily tobacco smokers vs nicotine 'vapers'



Source: https://www.athra.org.au/

3. From the graphs above, describe the patterns you see in the data for:

   (a) Lung cancer: _____

   (b) Tobacco smoking vs 'vaping': _____

4. What is the most likely reason for the conflicting trends in lung cancer cases and case rates?

   _____

5. What are some issues, regarding health outcomes, with only using the two graphs above to promote 'vaping' in youth?

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

207 Treating Non-infectious Disease

Key Idea: Treatment and management of non-infectious diseases enable reductions in symptoms, prolonging of life, and, in some cases, reversal of the disease. Research into the improved treatment of many diseases is ongoing.

Non-infectious diseases can be treated and managed with a variety of methods. These involve both medical intervention, and support for lifestyle choices that reduce risk factors. As many diseases in this category tend to be chronic, the benefits of treatment moderate an extensive and long-term demand on the health system. Reducing the severity of disease is also an important way to lessen the chance of comorbidities negatively compounding additional diseases.

Treating and managing type 2 diabetes

Blood glucose level monitoring

Monitoring is essential, as levels both too high and too low can create ongoing and immediate detrimental health impacts. Uncontrolled diabetes complications can include: heart and blood vessel disease, nerve damage, eye damage, difficulty in healing, and kidney disease.

Diabetes medication or insulin therapy

Insulin administration must be carefully linked to blood glucose monitoring. Excessive amounts of insulin can induce the same issues as low blood sugar, such as a neurological imbalance creating anxiety and confusion, even leading to coma.

Methods of treatment

Methods of prevention and control

Regular physical activity

Regular exercise improves blood glucose control and regulates body weight. This can prevent or delay the onset of type 2 diabetes, as a high BMI is one of the leading risk factors. Physical activity can also beneficially increase insulin sensitivity in cells.

Healthy diet

Regulating diet to avoid high carbohydrate foods can be one of the most effective means of controlling type 2 diabetes. In some cases, it can completely eliminate the symptoms and the requirement for insulin.

1. **Consequence chart:** Write an action in the centre of the table below (from methods of treatment of type 2 diabetes) – (a) and then write four possible consequences of this action in the surrounding boxes:(b) – (e); these can be negative or positive. Write consequences arising from these in the boxes on the outside of the table: (f) – (m); these might be directly attributed to treating the disease, or not.

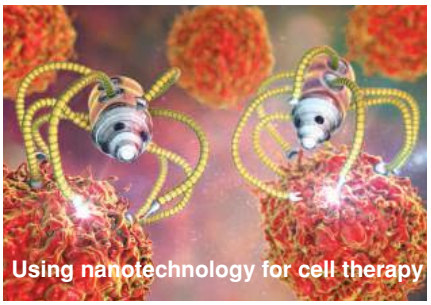
Further consequences	Consequences of action	Action taken	Consequences of action	Further consequences
(f)	(b)		(d)	(j)
(g)		(a)		(k)
(h)	(c)		(e)	(l)
(i)				(m)

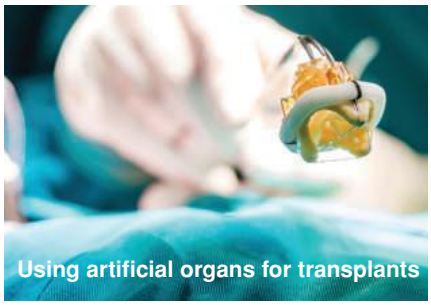
ISBN: 978-1-98-856655-9

Future treatments, management, and possible future research directions for non-infectious diseases

Research into future disease treatment and management is ongoing, and offers more efficient and effective possibilities. These include:

- Increasing the efficacy (ability to produce intended effect) of already available medications and treatments.
- Implementing new treatments e.g. utilising new technology such as gene therapy, cell therapy, or nanomedicine.
- Using artificial replacement organs or glands that have been 'grown' or built in the laboratory.
- Trialling new medicines, either manufactured, or from newly found species of plants, fungi, or bacteria.


Using nanotechnology for cell therapy


Using artificial organs for transplants


Research into new gene therapies

Research Investigation: Future directions and improvements in treating Type 1 or Type 2 diabetes

2. Use the information on the previous pages, and in the **BIOZONE Resource Hub**, to gain an understanding of diabetes and current treatments. Research at least THREE possible **future** directions or improvements in treatment of **type 1 and type 2 diabetes**, including a description, advantages, and disadvantages of each. Select ONE of your options below to prepare a class or group presentation in more detail, including diagrams.

Description of future treatment	(a)
Advantages of future treatment	(b)
Disadvantages of future treatment	(c)

Description of future treatment	(d)
Advantages of future treatment	(e)
Disadvantages of future treatment	(f)

Description of future treatment	(g)
Advantages of future treatment	(h)
Disadvantages of future treatment	(i)

ISBN: 978-1-98-856655-9

208 Are Epidemiological Methods Valid?

Key Idea: Epidemiology is the study of disease. In epidemiology we ask questions about causes, risk factors, distribution, and prevalence of disease. The validity of the method determines the quality of the research.

An epidemiological study begins with a thorough literature review of past and present research, and examines available data such as incidence and prevalence statistics from reputable sources. From these initial findings, researchers must generate a relevant and testable hypothesis or research question. A valid research method requires the design of a robust data collection plan, ensuring that the target population is correctly sampled. Any bias that may influence the results, such as lifestyle or comorbidities (other diseases or underlying factors) of the patients, is eliminated from the collected data, or accounted for, when sampling. The findings should be applicable to the larger population.

Epidemiology method classification

Observational

- Data is gathered and examined for possible trends in disease distribution, or to test a hypothesis.
- This type of epidemiological study does not directly interact with the disease progression.

Interventional

- A deliberate change is applied to both experimental and control groups to determine whether it has any impact on the disease.
- This type of epidemiological study interacts directly on disease progression, in a clinical setting.

Descriptive

- Data is collected using case studies, surveys, and cross-sectional studies from a population at one point in time. **There is no control group** (without the specific disease) to make comparisons with.
- 'What', 'who', 'where', and 'when' of the disease are the focus questions of this study.

Examined by the:

Person

Factors investigated:

- Demographics
- Lifestyle
- Comorbidities
- Occupation
- Genetic predispositions
- Behaviours

Place

Factors investigated:

- Economic status
- Climate
- Geography
- Population density
- Vectors
- Culture

Time

Factors investigated:

- Time since event
- Age
- Seasonality
- Physiological body cycles
- Calendar time

Analytical

- Data is collected in longitudinal cohort (group) (followed over time) studies, and case-control studies. **There is a control group** (without the specific disease) to compare against.
- 'How' and 'why' of the disease are the focus questions of this study.

Examined by the:

Host

Factors investigated:

- Immunity status
- Behaviour
- Personal traits
- Genetic predispositions

Environment

Factors investigated:

- Changes in transmission of disease agent
- Changes in development of disease agent

Agent

Factors investigated:

- Characteristics of disease source (agent) that increase, influence, or cause disease severity
- Biological, chemical, and physical aspects

ISBN: 978-1-98-856655-9

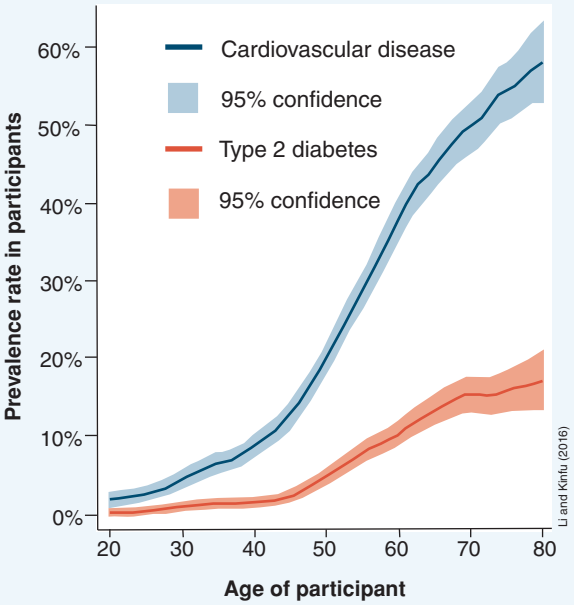
What is validity in epidemiology?

Validity asks the question: How legitimate are the results and conclusion of the study? Two components must be considered when evaluating the validity of an epidemiological method:

- **Internal validity:** Did the method enable the original question or hypothesis to be addressed correctly? Were all factors that could have impacted or distorted the results removed or accounted for? Internal validity measures the 'strength' of the data collection method, and how well variables, such as people, place, and time etc. are controlled.
- **External validity:** Did the method allow findings to be generalised and applied to other populations outside the study? External validity measures the appropriateness of the case or sample as a representative of a larger group, to allow correct inferences to be made.

Case study: Impact of socioeconomic and risk factors on cardiovascular disease (CVD) and type 2 diabetes in Australia

Prevalence of CVD and type 2 diabetes in 2013



- **Participants:** Data from individuals aged between 35 and 70 years was collected from a nationally representative longitudinal survey (HILDA) conducted in Australia between 2001 and 2013. In this survey, participants were approached annually, and data on any variables that may influence risk of type 2 diabetes and CVD were gathered.
- **Method:** Three sample groups were selected to compare with each other: 1) A cross-section of all participants. 2) All participants, but controlled for prior health conditions. 3) This group additionally adjusts for characteristics and risk profile, including comorbidity, of the respondents prior to onset of disease conditions.
- The following factors were accounted for in the method: relationship status, BMI, smoking status, alcohol use, frequency of physical exercise, and occupation.
- **Conclusion:** BMI and alcohol consumption show a more significant link to CVD in data group 3, when other factors were accounted for. Similarly, physical exercise and risk of type 2 diabetes show stronger correlation. However, socioeconomic factors showed almost no link to either disease once other factors were controlled in group 3.

1. (a) What type of observation epidemiology method is used in the case study? _____

   (b) Explain the reason for your choice of case study type: _____

   _____

2. The prevalence and age data from the most recent survey, taken in 2013, is self-reported by the participants; this is shown above in the graph. In earlier years of the survey, the data was collected by researchers, via interview. Which data set is likely to be more valid, and why might that be?

   _____

   _____

3. What would indicate that the data becomes less reliable as the age of the participant becomes greater?

   _____

4. Identify TWO factors that contribute to the **internal validity** of this study: _____

   _____

   _____

5. Discuss the graph above in terms of the **external validity** of this study, when applying results to the general population:

   _____

   _____

   _____

ISBN: 978-1-98-856655-9

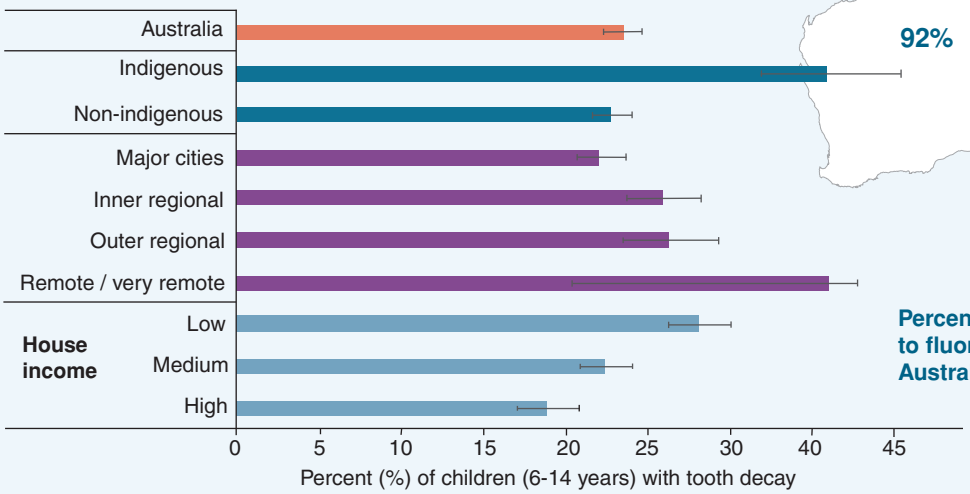
209 Benefits of Epidemiological Studies

Key Idea: Epidemiological studies offer multiple health benefits to a population and the scientific community.

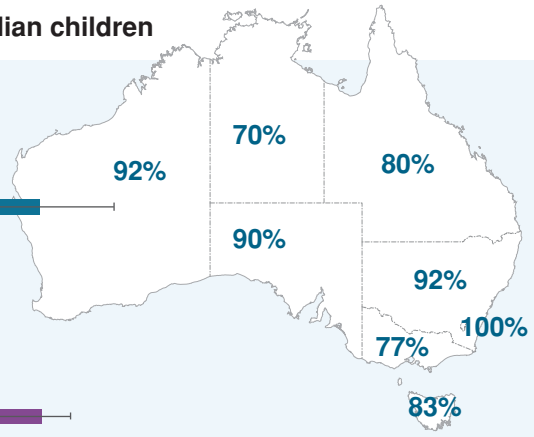
The measurement of risk factors in a population can help in the development of best practice interventions. Ongoing monitoring of disease incidence and prevalence in a community can inform targeted actions for timely prevention, treatment, and care. Findings from studies on agent and environment can be used in development of treatments. Finally, subsequent health programmes, public awareness programmes to reduce risk factors, and community services can be evaluated and modified to offer the most effective prevention and treatment.

Water fluoridation to prevent gum and tooth disease in Australian children

Proportion of children with permanent tooth decay (2012-2014)



Source: https://www.aihw.gov.au/reports/children-youth/australias-children/contents/healthy/dental-health after Ha DH et al 2016.



Percentage of population with access to fluoridation via drinking tap water, in Australian states.

- Fluoridated water is provided by all Australian states through public and tap water sources. Access to the water varies from state to state.
- Alternative means of water supply include private bores and water tanks; these are unlikely to contain sufficient fluoride.
- High-income houses are more likely to provide access to tap water to children compared to low-income households.
- Gender, indigenous status, or origin of parents did not impact children's water drinking patterns, when access is equal.

1. Construct a simple epidemiological study, ensuring validity, to test the hypothesis that fluoridation of water reduces tooth decay in children, investigating **access to tap drinking water** as a factor:

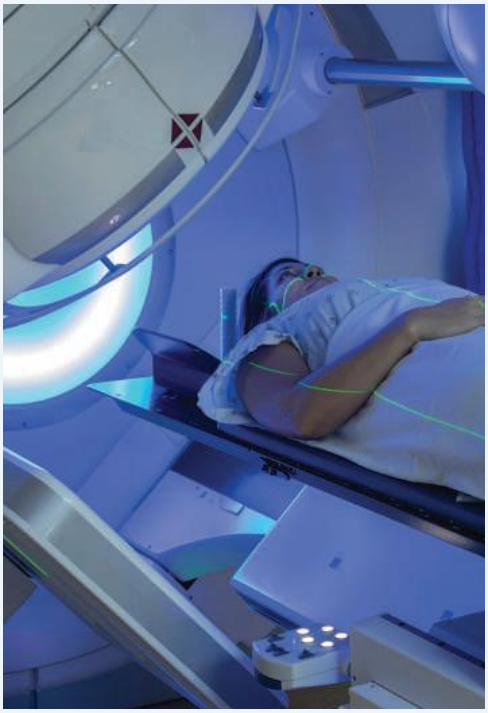
2. Explain some possible benefits of your epidemiological study in the following table:

Type of benefit	Specific benefit from your epidemiological study
How does your study target the segment of the population most impacted by tooth decay and disease?	(a)
How might your study assist with future scientific developments and treatments?	(b)
How might your study assist future health programmes and public awareness?	(c)

ISBN: 978-1-98-856655-9

210 Chapter Review: Did You Get It?

1. **Breast cancer** is the most common type of cancer in Australia and, in 2021, over 20,000 women (and some men) were newly diagnosed in the country. Current mainstream treatment of breast cancer can involve a combination of surgery, chemotherapy, radiotherapy, and hormone treatment. These treatments can cure the cancer, but also result in many debilitating side effects for the patient. Research ONE new possible treatment for breast cancer, describing how it might work to cure the cancer, or prolong the patient's life, and its advantages over current forms of treatment.



(a) Name of **new** breast cancer treatment:	
(b) How does this treatment work?	(c) What advantages does this treatment have over current breast cancer treatments?

2. What are two observational epidemiology study types? (a) _____ (b) _____

3. List some possible groups into which breast cancer incidence and prevalence can be divided, for comparison analysis:

_____

4. **Hypothetical case study:** Oestrogen (hormone) use by women who have undergone hysterectomy (uterus removal).
Hypothesis: Women who have undergone hysterectomy and subsequently treated with oestrogen have a lower incidence of breast cancer. (Note: no other criteria were used to select women for the study; cancer was medically diagnosed).
Results: Epidemiological studies yield conflicting results. After 7 years, breast cancer incidence was 23% lower in women treated with oestrogen in one study, but 30% higher in women treated with oestrogen in another study. The difference in these results (factors) may be explained by different screening of the women in these studies.

   Explain why the **validity of the method** used was considered **poor** in this study:

   (a) In internal validity: _____

   _____

   (b) In external validity: _____

   _____

5. Investment into breast cancer research is significant in Australia, with over $252 million in funding allocated between 2016 and 2018. What are some possible benefits of continuous epidemiological studies focused on breast cancer?

_____

_____

_____

_____

_____

_____

ISBN: 978-1-98-856655-9

CHAPTER 16

Prevention

Key terms
disease prevention
gene therapy
genetic engineering
secondary sources
spinal muscular atrophy
Spinraza®
Zolgensma®

Activity number

Inquiry question: *How can non-infectious diseases be prevented?*

Disease prevention methods

Key skills and knowledge

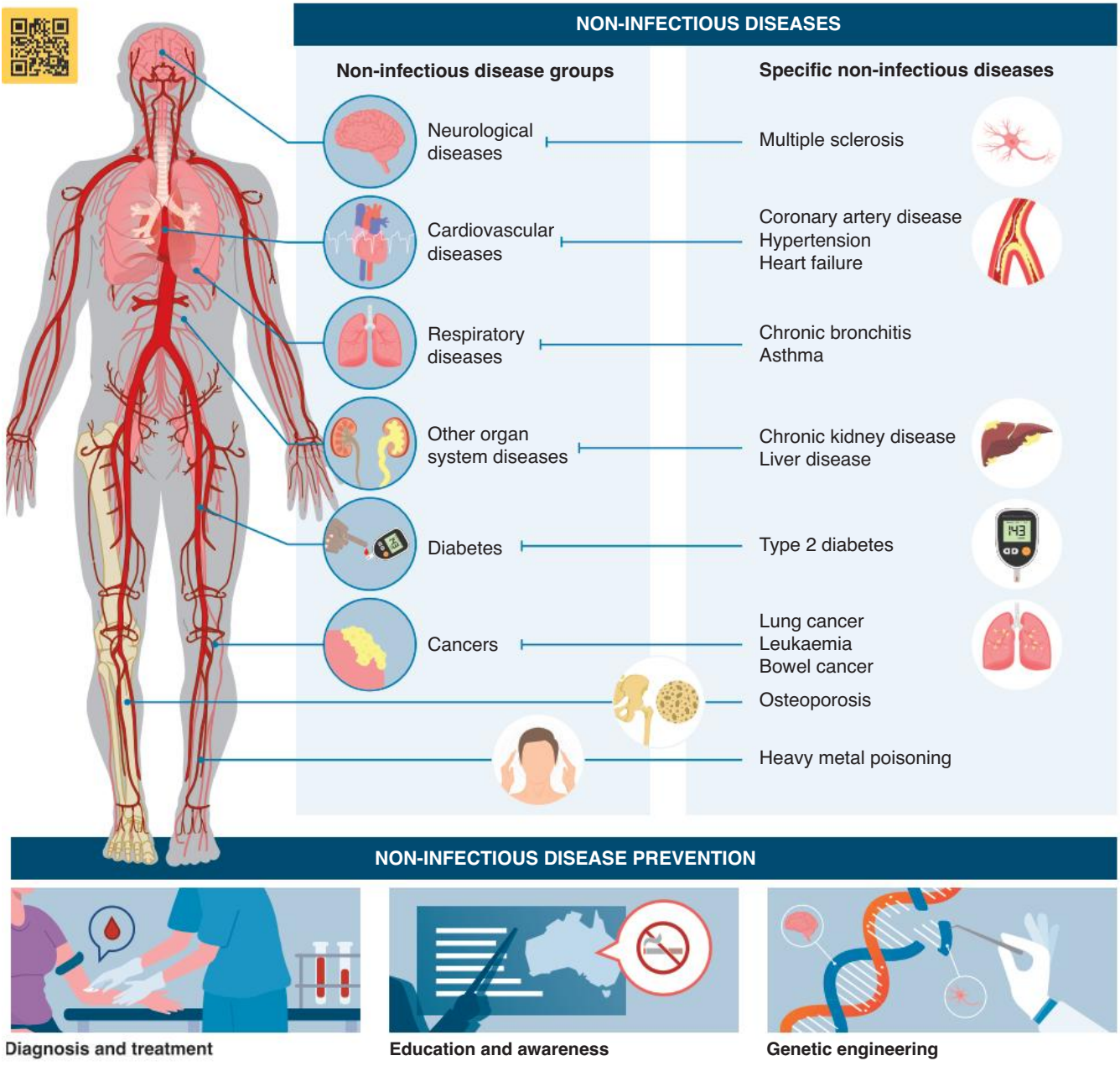
Investigating disease prevention methods

Key skills and knowledge

211 Investigating Disease Prevention Methods

Key Idea: Many current, key methods prevent incidence of non-infectious disease. These include genetic engineering, and community educational programmes and campaigns.

In order for a disease prevention method to be effective, there must be evidence that either the incidence or prevalence rate of the disease in a targeted community or population has decreased. Non-infectious diseases with a genetic component, either inherited or as a result of an agent such as cancer, may be prevented or cured by genetic engineering or gene therapy. Diseases that have manageable risk factors might benefit from a campaign or programme to reduce those factors, in a targeted population.



1. (a) Define genetic engineering: _____

_____

(b) Give one or more examples of non-infectious diseases that gene therapy has been used to prevent or cure:

_____

2. What educational programmes or campaigns are you aware of that have helped reduce risk-factors in preventing one or more non-infectious diseases? These may have been at school, community, national, or international level.

_____

_____

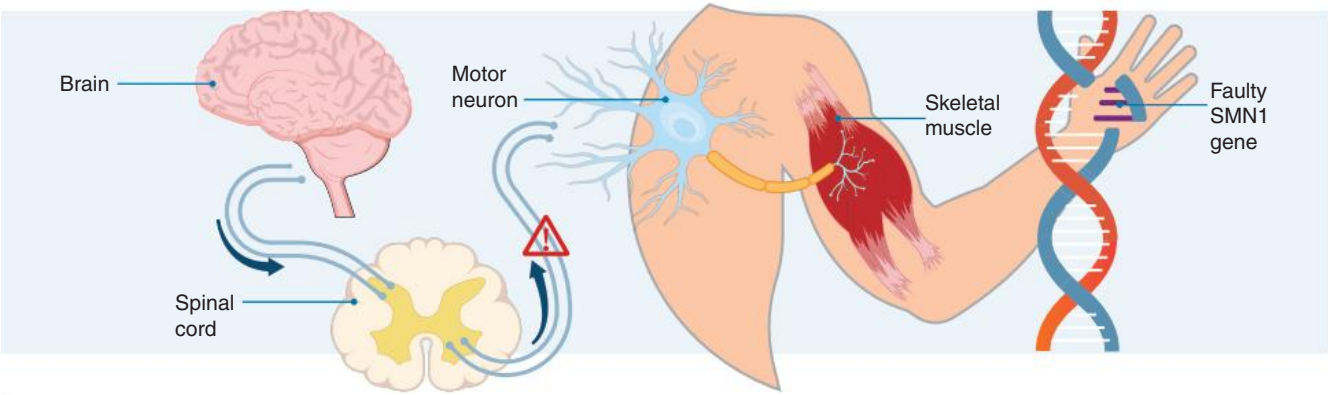
ISBN: 978-1-98-856655-9

Effectiveness of genetic engineering to prevent non-infectious disease

- **Gene therapy** is a specific type of genetic engineering that is used to prevent or treat a person affected by a disease. It alters or removes the relevant segment of DNA in a faulty gene, or adds a genetically modified replacement DNA section.
- If a potential disease is discovered early enough in an individual, usually by genetic screening of an infant, and the genetic abnormality can be corrected by gene therapy, then the disease and its associated symptoms may be entirely prevented.
- Effectiveness of this prevention method can encompass ease of use, permanence of treatment or prevention, the extent to which the disease is prevented, and applicability to a broad range of sufferers.

Case study - Gene therapy for spinal muscular atrophy (SMA)

- SMA is a rare genetic disorder that causes progressive destruction of motor neurons due to a mutation in a vital 'repair' protein gene (SMN1). Normally diagnosed by symptoms in babies, damage to motor neurons in the most severe cases results in inability to perform the most basic functions such as breathing and swallowing, and eventually causes death.



Spinraza®

https://thesocialmedwork.com/spinraza-nusinersen

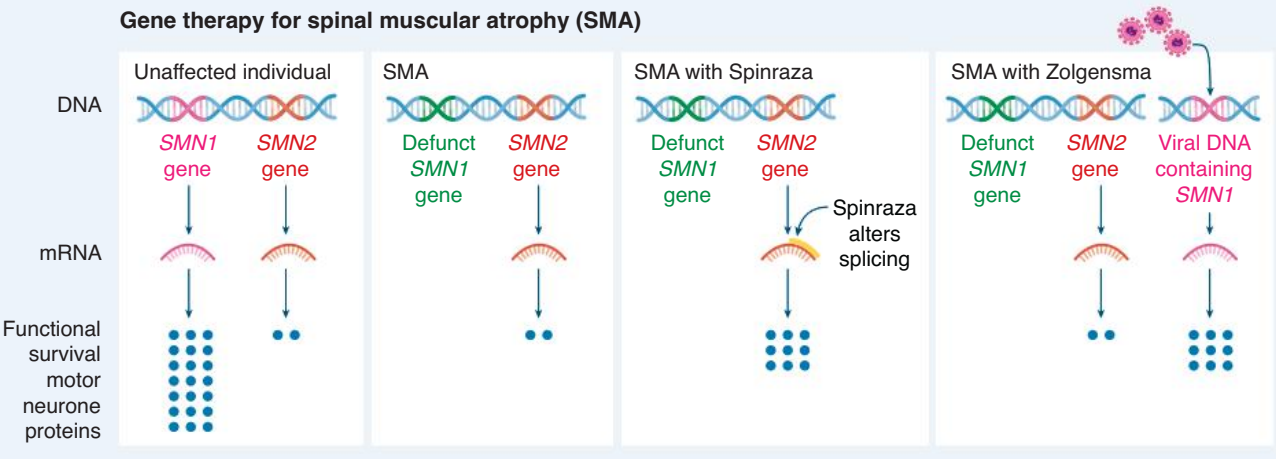
- An American biochemist helped to develop a gene therapy that delivers synthetic nucleotides directly into the cells. The amount of repair protein that was produced by a similar gene (SMN2) was increased, to compensate for the defunct SMN1 gene.
- **Positive considerations:** Disease symptoms can be alleviated in children to allow a longer life. The disease can even prevent the effects of SMA if the mutation is detected early enough in the fetus or baby through genetic screening.
- **Negative considerations:** Must be repeated every four months, as the genetic material is not permanently incorporated into the DNA. Cannot produce sufficient protein to completely restore nerve motor function. Marketed as Spinraza®, it is very expensive, costing around US$750,000 for the first treatment, and then US$375,000 annually.

Zolgensma®

https://www.npr.org/sections/health-shots

- Marketed as Zolgensma®, this drug is an SMA preventative for children under two years old. A new working copy of the faulty gene is introduced to the cells with a viral vector.
- **Positive considerations:** The treatment is a one-off, one hour long IV. It produces a much larger quantity of the missing repair protein, and some symptoms can be reversed or prevented entirely if administered before they develop.
- **Negative considerations:** This gene therapy is the most expensive medicine ever approved for use in the USA. It costs US$2.125 million for each treatment, and the high price makes its use prohibitive for many of the 1 in 6000 SMA babies born each year.

Gene therapy for spinal muscular atrophy (SMA)



ISBN: 978-1-98-856655-9

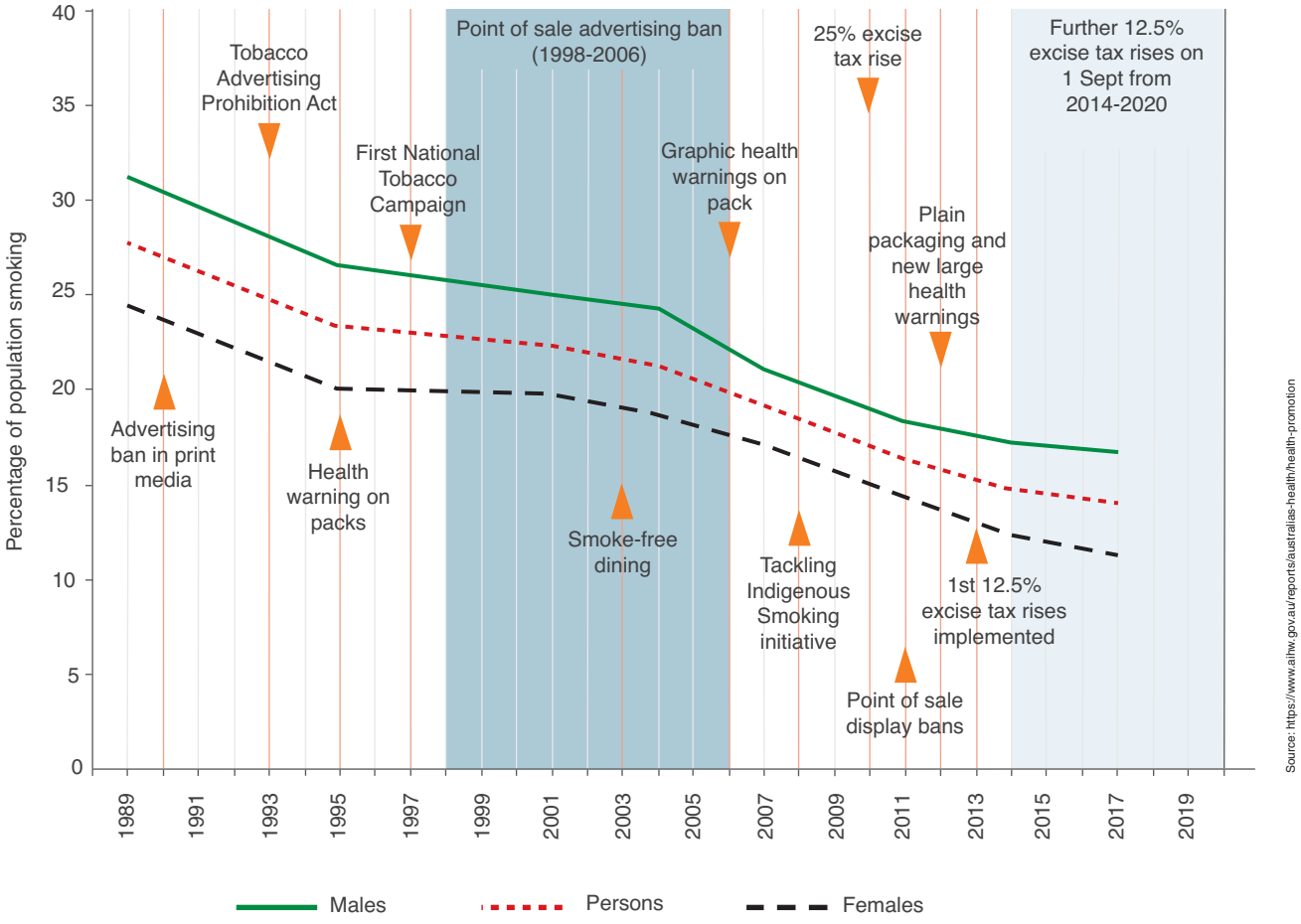
Effectiveness of educational programmes and campaigns to prevent non-infectious disease

- Health campaigns, education, and promotions focus on reducing the underlying risk factors that can lead to disease, rather than treatment. A combination of methods are used, including promotion of individual behavioural change towards healthy eating and physical exercise. National and state-wide policy change, such as restricting fast-food advertising to children, is also employed.
- 'Slip, slop, slap' was a memorable educational campaign to most Australians, in a bid to reduce skin cancer rates caused by exposure to excessive UV radiation, leading to sunburn.

Case study - Reducing tobacco use in Australia

- Because of a clear link between tobacco smoking and lung cancer, as well as other diseases, the Australian Government instigated a series of controls, campaigns, and public education initiatives to reduce tobacco use. Starting in 1989, these programmes focused on both the cessation of current smokers, as well as preventing the uptake of new smokers. Awareness of health impacts, new policy, and economic pressure, were key factors driving these successive measures.

Age-standardised daily smokers aged 18 and over, by sex and key tobacco control measures in Australia, 1989-2017



Source: https://www.aihw.gov.au/reports/australias-health/health-promotion

3. When considering the health of a population, many decisions have an ethical component, especially if the demand for a prevention or treatment exceeds the availability. What criteria would a doctor need to consider about an SMA patient when deciding on a course of treatment of Zolgensma®?

4. From the tobacco control campaign data above, discuss an event that you consider to be effective at producing the targeted result of reducing tobacco smoking in Australia, giving an evidence-based explanation for your choice:

ISBN: 978-1-98-856655-9

Secondary-sourced investigation report - evaluating the effectiveness of disease prevention methods

5. Your report will involve systematic scientific inquiry by planning a course of action and sourcing data and/or information from other people, including written information, reports, graphs, tables, diagrams, and images.

- You will select ONE non-infectious disease that uses **gene therapy or educational programmes and campaigns** as a means of prevention/treatment, excluding SMA and tobacco given as case studies on the previous page. An Australian context is preferable. Information to assist your selection can be found on the **BIOZONE Resource Hub.**
- Your report will include:
  - Introductory information about the non-infectious disease and method of prevention/treatment, which may include diagrams and images.
  - Evidence of secondary-sourced data showing the effect of the prevention/treatment on the disease and/or risk factors.
  - A written report on the effectiveness of the disease prevention/treatment using evidence-based statements linked to your sourced data. This might include sections of positive and negative impacts on the disease and risk factors, with a final summary paragraph on your assessment of the overall effectiveness of the prevention/treatment method in achieving its goals.
  - Use the tables below to **initially structure** your report. Complete your **final report** separately, in paper or digital format. A reference list is required.

Name of disease	(a)
Name of prevention/treatment	(b)

Description of disease	(c)
Description of how prevention/ treatment works	(d)

Secondary sourced data description (written/graphs/tables/images?)	Where was the data sourced? URL/organisation	What evidence-based claim does the data make about disease prevention/ treatment effectiveness?
(e)	(f)	(g)

Positive impacts of prevention/treatment on the disease and/or risk factors	(h)
Negative/neutral impacts of prevention/treatment on disease and/or risk factors	(i)
Evidence-based summary statements on effectiveness of disease prevention/treatment method	(j)

ISBN: 978-1-98-856655-9

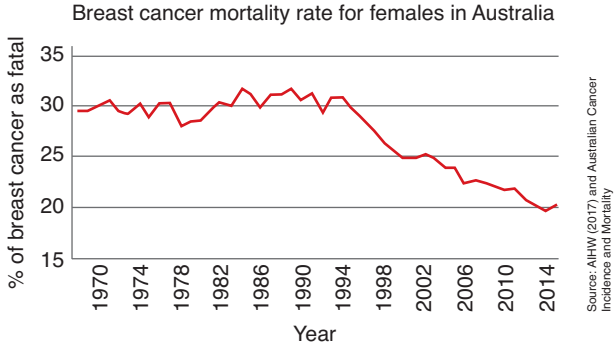
212 Chapter Review: Did You Get It?

1. What is the distinction between disease prevention and disease treatment?

2. Cardiovascular disease (CVD), resulting in heart failure and stroke, is currently the leading cause of death in Australia and accounts for 14% of the total disease burden. Risk factors for this disease include smoking, high cholesterol, diabetes, inactivity, and being overweight or obese. **Design a public health poster** that could be displayed in your local community centre or public areas, focusing on information about cardiovascular disease, and ways in which risk factors can be reduced by individual actions. You may choose to use the space below for a draft and complete digitally or on poster paper.

3. A series of promotional campaigns for free mammogram screening began in Australia, between 1991 and 1995, for women over 40, to reduce the mortality rate of breast cancer by preventing the fatal Stage IV breast cancer metastasis (spread) disease. In 2013, women between 70 and 74 were also specifically targeted.

   Use the graph and information above to make an evidence-based statement about the effectiveness of this disease prevention method.



ISBN: 978-1-98-856655-9

CHAPTER 17

Technologies and Disorders

Key terms

cochlea
cochlear implant
colour-blindness
countercurrent flow
dialysis
diffusion
hair cells
hypermetropia
LASIK
myopia
nephron
retina
xenotransplantation

Inquiry question: *How can technologies be used to assist people who experience disorders?*

Activity number

Ear, eye, and kidney disorders

Key skills and knowledge

Corrective technologies

Key skills and knowledge

Evaluating corrective technologies

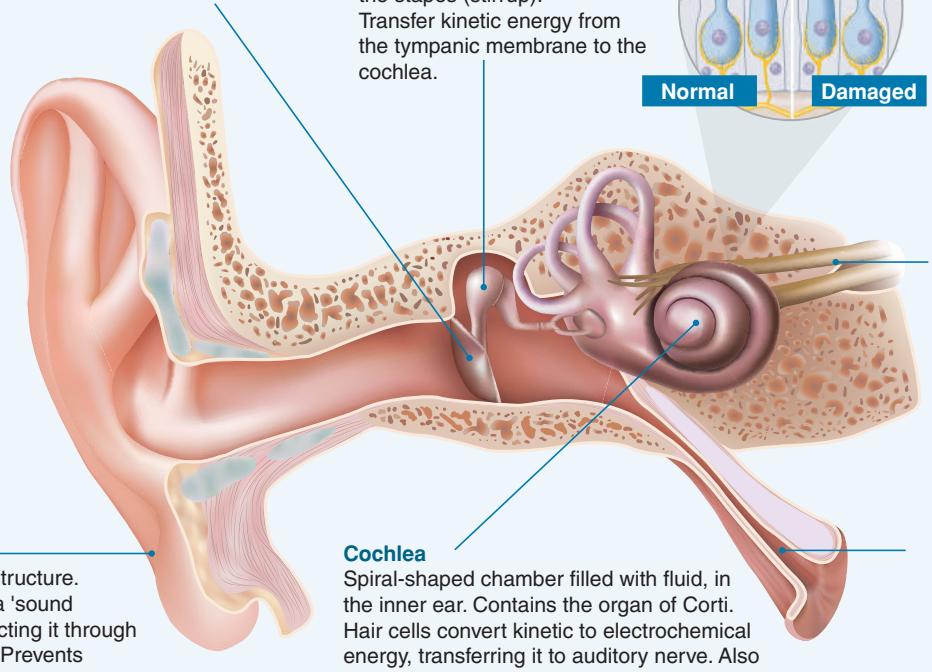
Key skills and knowledge

213 Disorders of Hearing

Key Idea: Hearing disorders can be classified as either conductive or sensorineural hearing loss.

The ear structure is divided into three sections: the **outer ear**, consisting of the pinna and ear canal; the **middle ear**, containing the eardrum and containing the ear ossicles (small bones), and the eustachian tube leading to the throat; and the **inner ear** containing the cochlea and the auditory nerve connecting to the brain. A range of hearing disorders can affect different structures within the ear. All hearing disorders are caused by some form of interference in the pathway by which sound stimulus reaches the auditory cortex of the brain, via the nervous system, as electrochemical signals.

The anatomy of the ear



Tympanic Membrane
Also called the eardrum, this is a thin, sensitive sheet between the outer and middle ear. The membrane oscillates in response to sound waves, converting sound into kinetic energy.

Ear Ossicles
Three small bones in the middle ear: the malleus (hammer), the incus (anvil) and the stapes (stirrup). Transfer kinetic energy from the tympanic membrane to the cochlea.

Organ of Corti
Organ on the inner surface of the cochlea. Contains sensory receptors called hair cells. Hair cells convert sound vibrations into electrochemical signals that are transmitted to the brain through the auditory nerve.

Auditory Nerve
Nerve fibres connecting ear to brain. Transmits signal to the auditory cortex in the brain, to be processed as sound.

Pinna
Outer visible structure. Functions as a 'sound collector', directing it through the ear canal. Prevents objects from entering ear.

Cochlea
Spiral-shaped chamber filled with fluid, in the inner ear. Contains the organ of Corti. Hair cells convert kinetic to electrochemical energy, transferring it to auditory nerve. Also registers body position, aiding balance.

Eustachian tube
Connects middle ear and throat. Equalises pressure to maintain eardrum position.

Hearing loss

Types of hearing loss are divided into: conductive, involving sound wave interference between outer and middle ear; and sensorineural, concerning issues of the inner ear or auditory nerve.

Causes of conductive hearing loss	Causes of sensorineural hearing loss
• Ear infections in outer and middle ear. • Glue ear. • Excessive ear wax and foreign bodies in ear canal. • Mis-shaped structures in outer and middle ear. • Otosclerosis - a genetic disorder that hardens the stapes bone due to extra growth.	• Progressive damage to cochlear hair cells in the organ of Corti due to old age, after a lifetime of noise. • Excessive or repeated loud nose exposure, causing damage to hair cells. • Excessive loud noise, damaging auditory nerve such that sound is not transmitted correctly to the auditory cortex. • Genetic disorders, including the CABPP2 gene mutation, that affects transmission from hair cells.

1. List the predominant **energy form** present in each structure during the process of hearing:

   (a) Ear canal:_____ (b) Ear ossicles:_____ (c) Auditory nerve: _____

2. Explain how persistent loud noise can cause hearing damage, linking to specific structures in the ear:

   _____

   _____

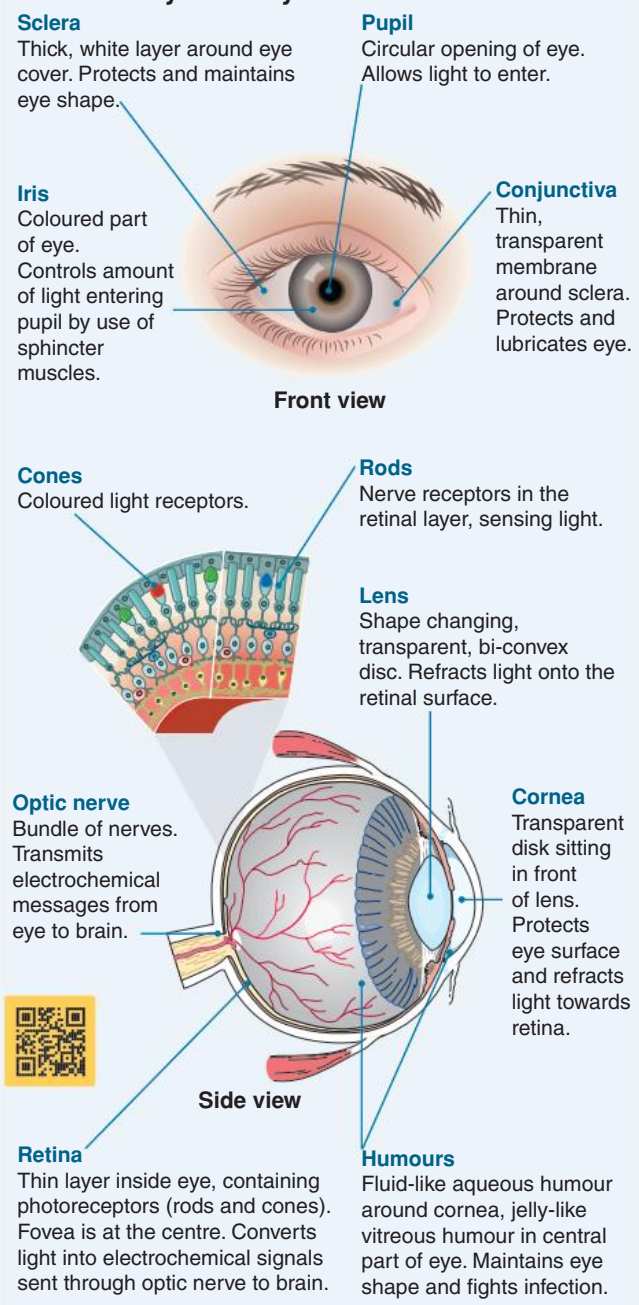
ISBN: 978-1-98-856655-9

214 Disorders of Vision

Key Idea: Vision disorders can be inherited, or can arise from environmental damage. They affect a range of structures and their functions in the eye.

The eye is the organ responsible for vision, and a multitude of different structures work together to process light energy into coherent electrochemical signals which are sent to the visual cortex of the brain. Total blindness from birth can be due to many factors, including an inherited genetic disorder, premature birth, and nutritional deficiency in the uterus. Sight disorders, such as colour-blindness, are also inherited. Many other disorders, such as long or short sightedness, and cataracts, become more pronounced as a person ages.

The anatomy of the eye



Vision disorders

Types of vision loss can be divided into those affecting the entry of light and how it focuses onto the retina, and those affecting light conversion into electrochemical signals.

Causes of vision disorder in the lens and cornea

- **Myopia**, or short-sightedness, is often caused by a cornea with high curvature (more rounded), which refracts (bends) the light entering the eye and causes it to focus before reaching the retina. Images are blurred. This disorder can be both genetically inherited and arise from excessive eye strain.
- **Hypermetropia**, or long-sightedness, is a condition where the light entering the eye is focused behind the retina, also causing blurred images. This disorder is often the result of reduced curvature of either the cornea or the lens, or a misshapen eye ball.
- **Cataracts** scatter light due to a 'cloudy' lens. Proteins in the eye build up and block the straight passage of light through the lens. This condition is common as a person ages but can also be caused by a comorbidity such as diabetes, nutritional deficiency, or environmental exposure to bright sunlight.

Causes of vision disorder in the retina

- **Colour-blindness** is usually due a malfunction of the pigment-containing nerve receptors, known as rods and cones, in the retina. It can also be caused by damage to the optic nerve or visual cortex. There are three types of cone cells: red, blue, and green, each detecting a different visible wavelength range or colour.
- **Deuteranomaly** occurs when the green cone does not work well, and is the most common form of colourblindness; it is most often found in men. It causes images to appear redder, and makes purple and blue indistinguishable from each other.
- **Cone monochromacy** is due to malfunction in two or more coloured cones, and colours are difficult to distinguish. This condition may also cause myopia and nystagmus (uncontrolled eye movement).

1. Colour-blindness can be classified according to the affected coloured cone cells. Use an online search to complete the chart below to indicate which coloured cone cells function correctly (write yes) and those which don't (write no).

Type of colour-blindness	Red cone	Blue cone	Green cone
Protanopia	(a)		
Tritanopia	(b)		
Deuteranopia	(c)		
Achromatopsia	(d)		

ISBN: 978-1-98-856655-9

215 Disorders of Kidney Function

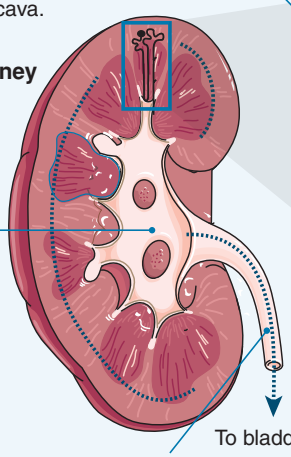
Key Idea: Kidneys play a vital role in the excretory system and various diseases, either inherited, nutritional, or environmental, can impact their ability to function.

Kidneys contain around a million nephrons. These are the main structures that filter the blood, remove waste, and balance ion levels, while also reabsorbing excess water. Disorders that prevent proper functioning of the kidneys can allow harmful build-up of waste, blood glucose, and toxins in the blood. Damage to the nephrons, by increased blood pressure, kidney stones, or cysts, can result in kidney failure, a fatal condition that is normally only controlled by dialysis. Sufferers may eventually require a kidney transplant.

The anatomy of the kidney

Renal artery
A pair of arteries branching directly off the aorta. One connects to each kidney. They continue to subdivide and form a mesh of capillaries at each separate nephron.

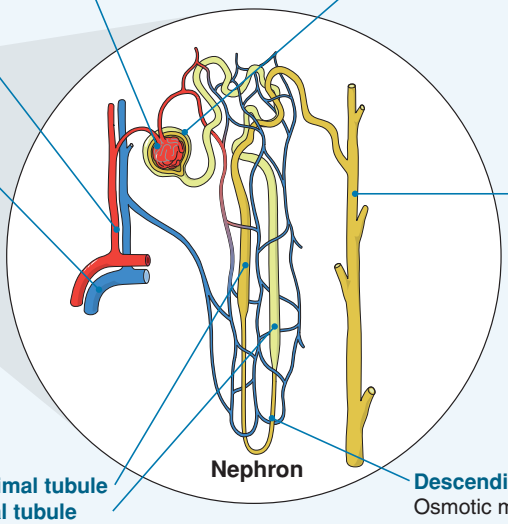
Renal vein
Returns filtered blood to the heart, via the inferior vena cava.



Ureter
Collects urine from the renal pelvis and connects to the bladder. Urine is moved by smooth muscle peristalsis (contraction waves).

Glomerulus
Bundle of compacted, high pressure, capillaries. Blood filtering of glucose, ions, and water occurs here. Blood cells and protein do not pass through, due to their large size.

Bowman's capsule
Surrounds the glomerulus, draining the filtrate from the blood and transporting it towards the collecting duct.



Collecting duct
Collects and carries urine from each nephron to the ureter and bladder for excretion from the body.

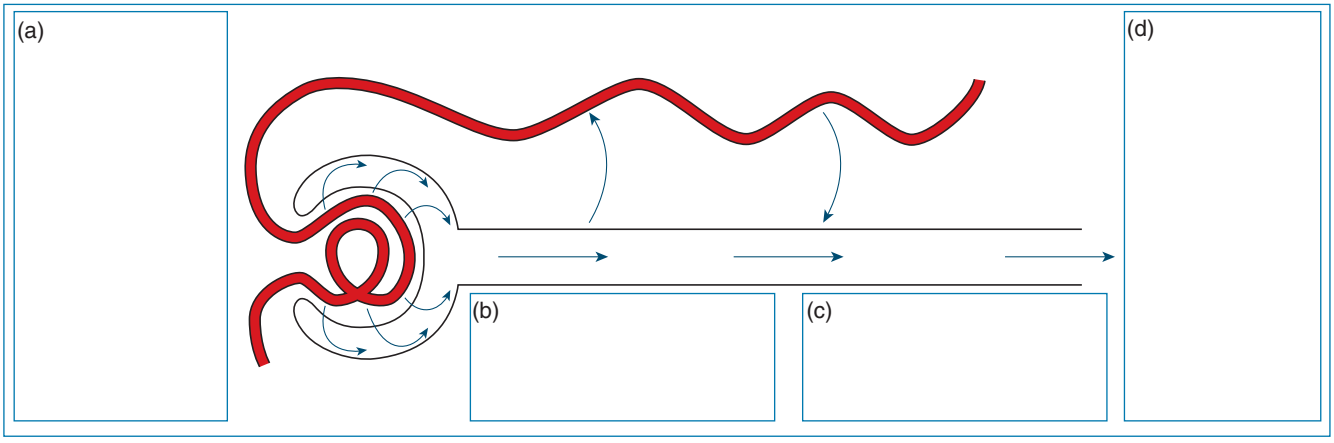
Proximal tubule
Distal tubule
Both are regions where active and passive transport carry important substances back into the blood in the process of reabsorption. Urea moves from the blood into tubules.

Descending loop of Henle
Osmotic movement of water from the glomerular filtrate into the blood vessels happens here, as well as passive transport of sodium ions.

Kidney function disorders

Genetically inherited kidney disorders	Non-genetic kidney disorders
• **Polycystic kidney disease** causes fluid filled sacs to build up in the nephrons, altering their shape. Eventually, without treatment, this leads to kidney failure. Two main forms of this disease exist: one is autosomal dominant, and the presence of only one mutated gene is required for the condition to be present; the other is autosomal recessive and, if both parents are carriers, their offspring have a 25% chance of inheriting the disease.	• **Chronic kidney disease** is often caused by hypertension (high blood pressure) that damages nephrons. Type 1 and type 2 diabetes also cause progressive damage, due to uncontrolled blood glucose. • **Kidney stones**, often due to excessive salt in the diet, crystallise and create pressure damage, sometimes leading to kidney failure.

1. Annotate parts of the diagram to indicate the direction of movement of water, ions, urea, and glucose in the nephron.



ISBN: 978-1-98-856655-9

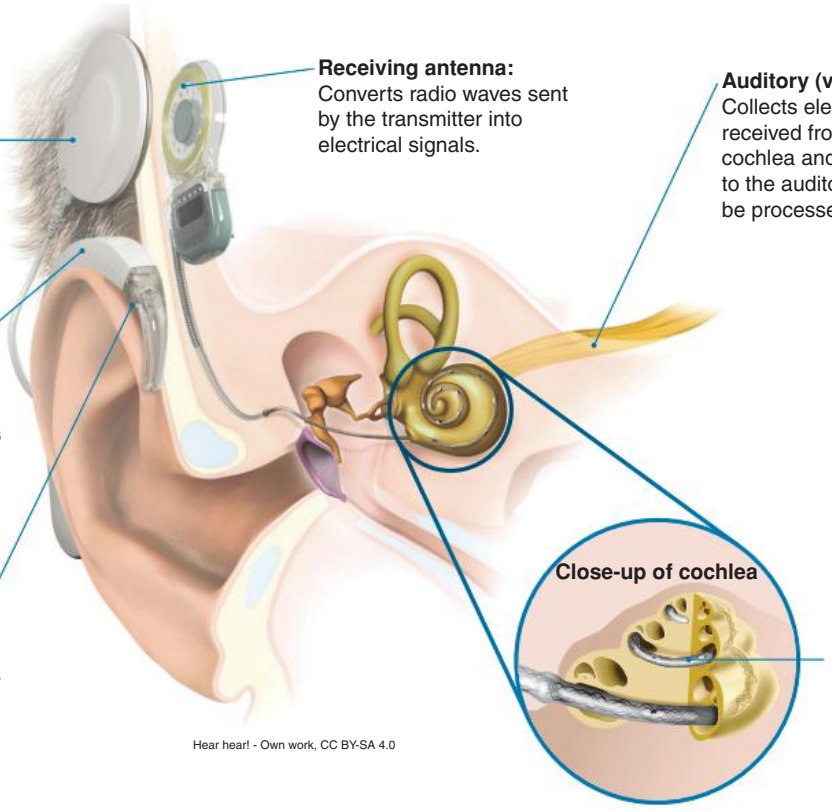
216 Correcting Disorders Through Technology

Key Idea: The symptoms of some disorders can be alleviated or eliminated by medical technology.

Technology can be used as a treatment for a number of disorders, including those of the eye, ear, and kidney. Hearing loss can be improved with external hearing aids, surgically embedded cochlear implants, and bone conduction implants. Vision problems of the eye can be corrected with glasses incorporating a specially fitted lens, or, more permanently, with laser eye surgery (LASIK), and cell therapy. Finally, dialysis is a common temporary medical procedure for kidney disorders, with kidney transplants used as a more permanent treatment.

Cochlear implant

While a hearing aid can be used to amplify sound waves if the cochlear hair cell receptors are partially damaged, it will not work at all if the hair cells are completely non-functioning. The cochlear implant is surgically inserted and completely bypasses the hair cells. It transmits information received from sound waves directly to the auditory nerve that connects to the brain. This technology, while expensive, can restore hearing to certain groups of people that would otherwise be deaf.



Transmitter: Converts signals from the speech processor into radio waves that are then sent on to the receiver.

Receiving antenna: Converts radio waves sent by the transmitter into electrical signals.

Auditory (vestibulocochlear) nerve: Collects electrochemical signals received from different parts of the cochlea and sends them, eventually, to the auditory cortex of the brain to be processed.

Speech processor: Filters out unnecessary noise received from the microphone, and converts sound energy into electrical energy.

Microphone: Detects any external sound waves reaching the ear.

Electrode array: Relays the electrical signal received from the receiving antenna to stimulate different areas of the cochlea. There are usually 22 electrodes in the cochlea. The higher the frequency and pitch perception of the original sound, the further into the spiral that the electrode will stimulate the nerves.

1. The initial stimulus received by the cochlear implant microphone undergoes a number of energy transformations before reaching the auditory cortex of the brain. Write the energy transformation (if one exists) at each stage of the pathway.

1. Microphone	2. Speech processor	3. Transmitter	4. Receiving antenna	5. Electrode array
(a)	(b)	(c)	(d)	(e)

2. What structure and function in the ear does the **electrode array** replace, and explain in detail how it achieves that?

ISBN: 978-1-98-856655-9

Corrective glasses for myopia and hypermetropia

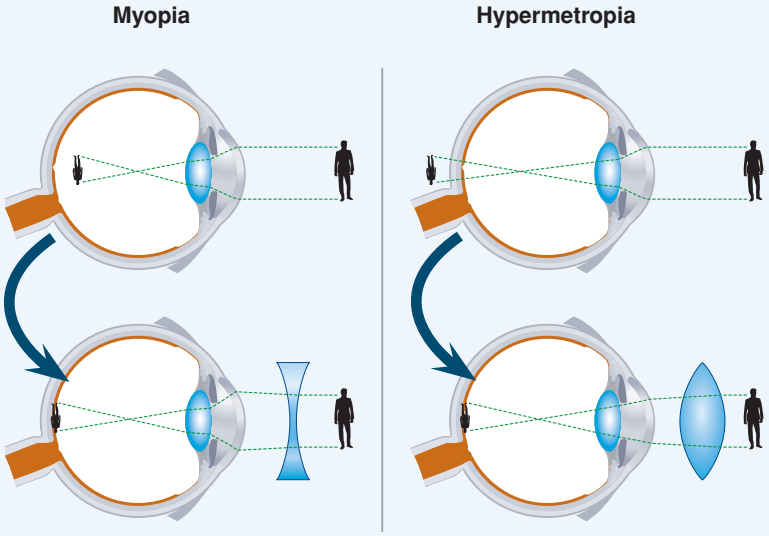
- The degree of refraction occurring at each surface of the eye is precise. Light rays reflected from an object 6m or more away are nearly parallel to one another; those reflected from near objects are divergent. The light rays must be refracted differently in each case so that they fall exactly on the central fovea of the retina. This is achieved by adjustment of the shape of the eye lens by the surrounding ciliary muscle and is known as accommodation. If the lens is unable to change shape correctly, or the eyeball shape is incorrect, corrective lenses can be used to restore normal vision.

Short sightedness (myopia)

- Myopia results from an elongated eyeball or a thickened lens. Left uncorrected, distant objects have a point of focus in front of the retina and appear blurred.
- To correct myopia, concave (negative) lenses are used to move the point of focus backward to the retinal fovea.

Long sightedness (hypermetropia)

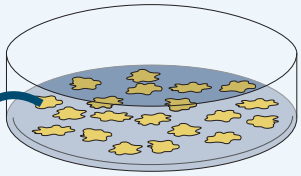
- Long sightedness results from a shortened eyeball or from a lens that is too thin. Left uncorrected, light is focused at a point that would be behind the retina and near objects appear blurred.
- Mild or moderate hypermetropia, which occurs naturally in young children, may be overcome by accommodation. In more severe cases, corrective lenses are used to bring the point of focus forward to produce a clear image. This is achieved using a convex (positive) lens.



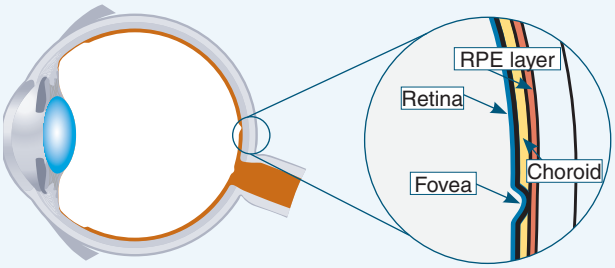
Cell therapy - Stem cells for Stargardt's disease

Stargardt's disease is an inherited form of juvenile macular degeneration, which is loss of the central visual field of the eye. The disease is associated with a number of mutations and results in dysfunction of the retinal pigment epithelium (RPE) cells, which nourish the retinal rod and cone cells and protect the retina from excess light. Damaged RPE causes a deterioration of the photoreceptor (rod and cone) cells in the central portion of the retina, and progressive loss of central vision. This often begins between ages 6 and 12 and continues until a person is legally blind. Trials using stem cells have obtained promising results in treating the disease.

1. Human embryonic stem cells (hESC) are cultured in the lab to develop into retinal pigment epithelium (RPE) cells.



2. The RPE cells are injected just below the retina of the eye and above the choroid (the layer containing the blood vessels).
3. The RPE cells develop and replace the patient's damaged RPE cells, restoring vision.



3. LASIK eye surgery permanently changes the shape of the cornea. What are the key differences of this technology in comparison to wearing corrective lenses?

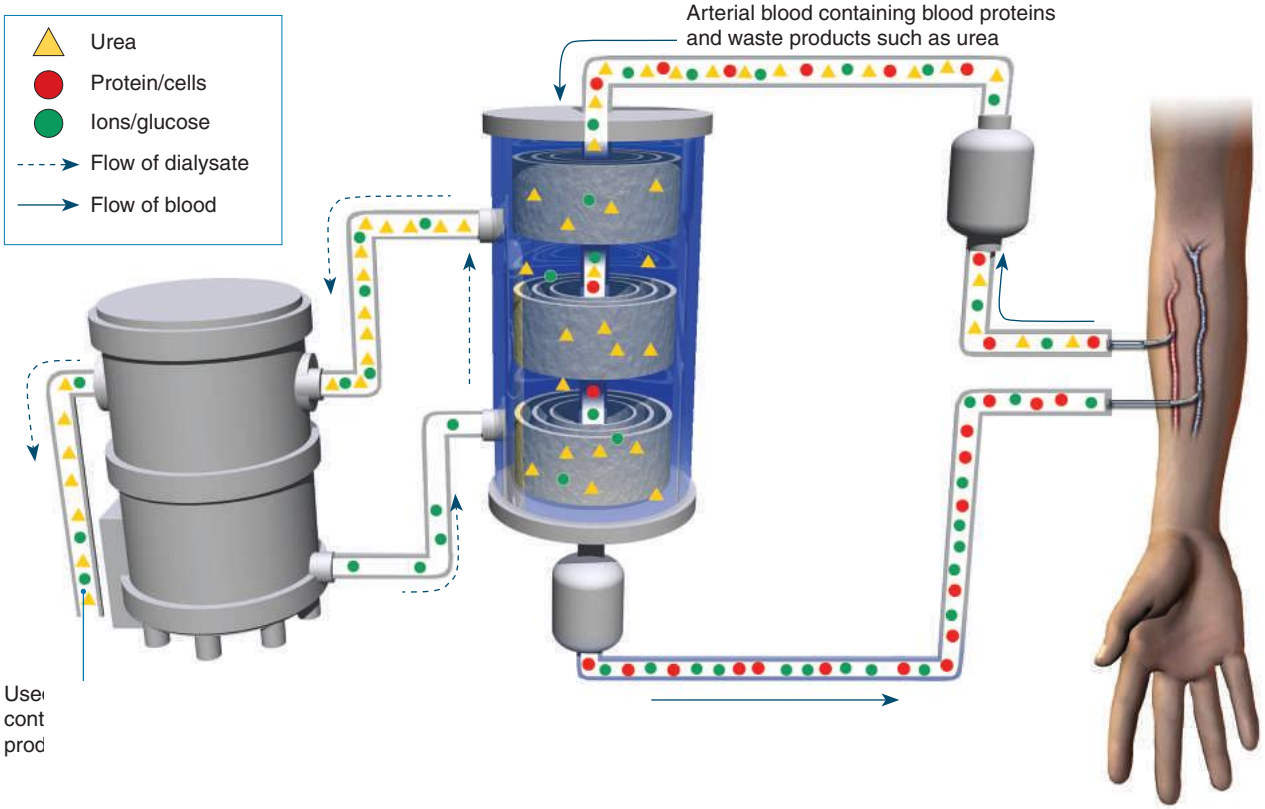
4. Why would LASIK eye surgery or corrective lenses **not** be useful to a person with Stargardt's disease?

5. Embryonic stem cells, used in the cell therapy for Stargardt's disease are **pluripotent.** Define this word:

ISBN: 978-1-98-856655-9

Dialysis for kidney failure

- A kidney dialysis machine acts as an artificial kidney, removing waste such as urea from the blood. When the kidneys do not function properly, waste products build up in the body, and medical intervention is required to correct the problem. A dialysis machine is used when the kidneys fail, or when blood acidity, urea, or potassium levels increase above normal. Blood flows through a system of tubes composed of partially permeable membranes.
- Dialysis fluid (dialysate) has a composition similar to blood except that the concentration of wastes is low. It flows in the opposite direction (countercurrent flow) from the blood on the outside of the dialysis tubes. Consequently, waste products like urea **diffuse** from the blood into the dialysis fluid due the concentration gradient.
- Dialysis can be ongoing, or can be used to allow the kidneys to rest and recover from injury, the effects of drugs, or other metabolic disturbances. Treatment is 3-5 hours each time, and is usually required three times a week.



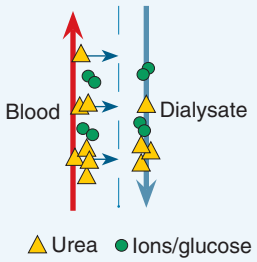
6. In kidney dialysis, explain why the dialysing solution is constantly replaced rather than being recirculated:

7. Explain why ions such as potassium and sodium, and small molecules, e.g. glucose, do not diffuse rapidly from the blood into the dialysing solution along with the urea. Use the **countercurrent flow** diagram (right) to help you:

8. Explain why the urea passes from the blood into the dialysing solution:

9. Give a reason why the dialysing solution flows in the opposite direction from the blood:

Countercurrent flow



Countercurrent flow occurs when two fluids move past each other, separated by a semi-permeable membrane. Substances move from high concentration to low concentration (gradient) by the process of diffusion.

ISBN: 978-1-98-856655-9

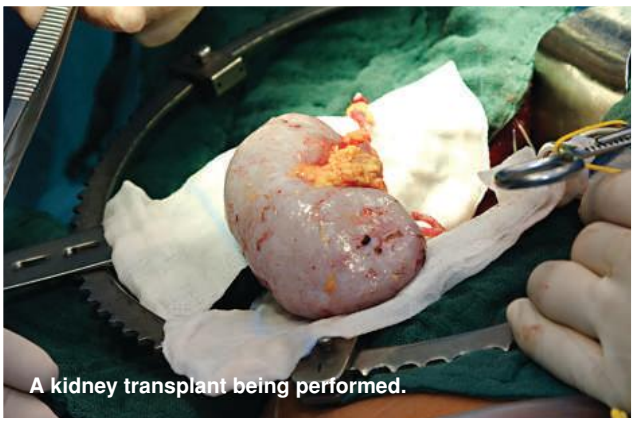
217 Evaluating Corrective Technology

Key Idea: The effectiveness of corrective technologies to improve vision, hearing, and kidney function can be evaluated to ensure that the correct procedure or device is selected.

The type of technology selected to improve or eliminate disorders is dependent on a number of factors, including the type and severity of the disorder, permanence of treatment, availability and affordability of the technology, and personal choice of the patient. Medical practitioners and patients need to consider all of these factors, and evaluate which technology would be the most suitable in each individual case. Newly developed technologies ensure that a wider range of options becomes available in future.

Kidney transplant

- Transplantation of a healthy kidney from an organ donor is the preferred treatment for end-stage kidney failure. The organ is usually taken from a person who has just died, although kidneys can also be taken from living donors. The damaged kidneys are left in place and the new kidney is transplanted into the lower abdomen (below).
- As long as recipients comply with medical requirements, including correct diet and medication, over 85% of kidney transplants are successful and achieve full functionality.



A kidney transplant being performed.

- Two major problems are associated with kidney transplants: lack of donors and tissue rejection. Cells from donor tissue have different antigens to those of the recipient, and the body's immune system will attack the new kidney.
- Tissue-typing and the use of immunosuppressant drugs help to decrease organ rejection rates. In future, the transplant of genetically modified organs from other species may help to solve the problems of supply and immune rejection.

1. What factors are considered when selecting a patient with kidney failure to receive a kidney transplant?

2. Dialysis is usually an effective method to replace kidney function, yet there is still a large demand for kidney transplants. Why might a patient prefer a kidney transplant over dialysis, despite the invasive surgery and difficulty in securing a donor?

In late 2021, a trial kidney transplant took place using a genetically modified pig kidney inserted into a human body. The kidney continued to function properly until the investigation was stopped 72 hours later.

When cross-species transplants are used, the procedure is called **xenotransplantation**.

3. When evaluating new technology, scientists and medical practitioners need to consider many factors, including ethics. Bullet point what you might consider the **pros and cons** of xenotransplantation to treat kidney failure, then make a final **summary statement of your position** on the use of this technology. Use a **class debate** format to share ideas.

Pros	(a)
Cons	(b)
Your position	(c)

ISBN: 978-1-98-856655-9

Corrective technology for hearing loss

Hearing aids

Hearing aids are small, battery-powered devices that fit inside the ear canal and amplify sounds. A microphone detects sound waves, which are converted to a digital signal. These signals are then amplified and sent to the ear through a speaker.

Hearing aids are useful when the hair cells still have some partial function, as the amplified signals boost the threshold for being stimulated by sound level.

A man with a fitted hearing aid

Hearing aids are relatively inexpensive in comparison to more invasive surgical technologies such as cochlear and bone conduction implants. However, they must constantly have batteries charged or replaced, and can be easily lost or damaged when out of the ear.

Once the hair cells cease to function, either through ageing, damage due to loud noise, or through an inherited genetic disorder, the hearing aids become ineffective.

Bone conduction implant

Sound waves normally travel through to the cochlea via the ear canal, tympanic membrane, and ear ossicles. If any of these are damaged or non-functional, they can be bypassed with a bone conduction implant.

These devices are surgically implanted in the bone of the skull behind the ear and receive sound vibrations that are detected by an external microphone. They transfer sound by bone vibration directly to the cochlea.

A man with a bone conduction implant

Josabeth, CC BY-SA 3.0

Bone conduction implants are permanently attached to the body. However, they do require some functionality of the hair cells in order to improve hearing. Because of this, they are not a suitable technology if all function has been lost in the hair cells.

A detachable version of this device is also available, and offers similar advantages and disadvantages to hearing aids as a non-surgical option.

4. Hearing aids, cochlear implants, and bone conduction implants all assist in correcting hearing loss. The selection of suitable technology depends on the area of damage or dysfunction in the ear. Complete the chart below to show which area, and the extent of damage if appropriate, that is compensated for by each device:

Technology	Associated area of ear damaged
Hearing aids	(a)
Bone conduction implant	(b)
Cochlear implant	(c)

5. A patient has been diagnosed with a vision disorder and is keen to receive LAZIK surgery because she was told her vision could not be corrected with glasses. Would this patient be a suitable candidate for LAZIK? What reason would you give for your answer?

6. Scientists have started to research and develop the technology for a more effective and portable kidney dialysis device that can be carried around. What are some factors that they would have to consider when designing this device?

ISBN: 978-1-98-856655-9

218 Chapter Review: Did You Get It?

1. Sort the following terms of eye, ear, and kidney structures into the correct boxes below:

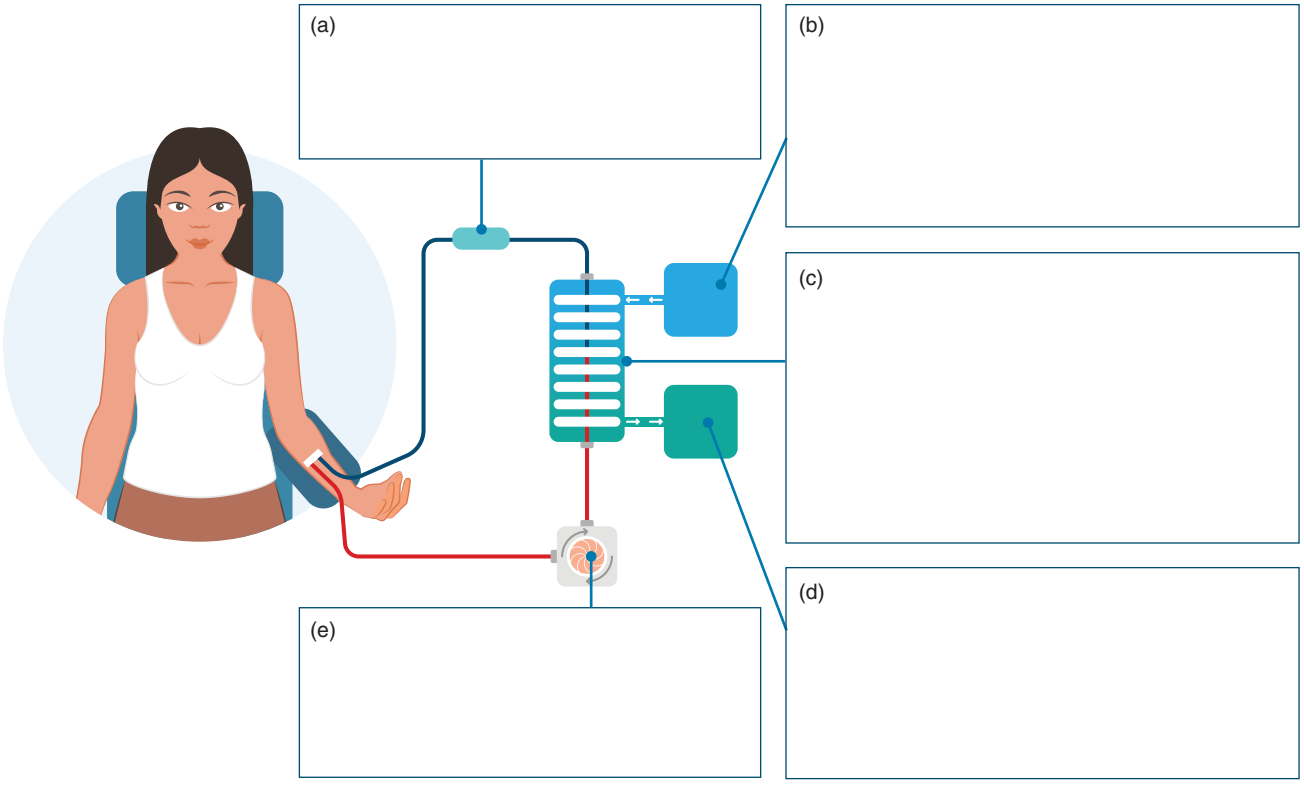
pinna conjunctiva sclera descending loop of Henle cochlea hair cells tympanic membrane eustachian tube glomerulus organ of Corti retina Bowman's capsule cornea malleus distal tubule renal pelvis

Eye structures	Ear structures	Kidney structures
(a)	(b)	(c)

2. Kidney failure can be diagnosed by blood tests and urine analysis. Levels of the protein creatinine (a breakdown product of metabolism) in blood and urine indicate how well the kidneys are working. In normally functioning kidneys, most of the creatinine is filtered out of the blood into the urine. If the kidneys are damaged, the amount of creatinine in urine decreases, while its level in the blood increases. Discuss the importance of these diagnostic tests in evaluating a suitable kidney recipient, finding a suitable donor, and monitoring the success of the kidney transplant:

3. Discuss the advantages and disadvantages of using the cochlear implant over hearing aids for **partial hearing loss**:

4. Label the following diagram of haemodialysis (dialysis of the blood) with the correct terms for the part indicated, and the purpose of that labelled part:



ISBN: 978-1-98-856655-9

219 Synoptic Assessment: Module 8

1. A swimmer has stepped out of the pool and feels cold. Discuss how homeostasis mechanisms enable the swimmer to maintain her body temperature?

- Name the specific receptor, control centre, and effector(s) in temperature homeostasis and explain their general role.
- Link the specific components of the body to the response they produce to 'warm' the body.
- Explain how the nervous system is able to transmit information in the process of temperature homeostasis.
- Explain the importance of a negative feedback loop in temperature homeostasis.

2. Plants in pots beside the pool have not been watered for a while and are under water stress. What is the homeostatic response of the plants to maintain water balance?

- Explain the role of abscisic acid in maintaining plant water homeostasis.
- Describe the process and purpose of transpiration.
- Describe the structure of the stomata, using correct terminology.
- Link the stomatal structure to the process of transpiration and control of water balance.
- Explain how water potential controls stomatal opening and closing.

ISBN: 978-1-98-856655-9

3. Type 2 diabetes is a non-infectious disease that interferes with the homeostatic control of blood glucose in the body. Discuss the effect type 2 diabetes has on the body, including causes, treatments, and possible prevention methods.

    - Describe the type 2 diabetes disease and the common symptoms experienced by an individual.
    - Explain the homeostasis mechanism of blood glucose control. You may wish to draw a labelled diagram.
    - Explain how type 2 diabetes disrupts the blood glucose homeostasis control.
    - Describe the risk factors that increase the chance of developing type 2 diabetes.
    - Explain how ONE current treatment of type 2 diabetes controls or eliminates the disease.
    - Link ONE possible prevention strategy to reduce the incidence of the disease, explaining how it could be effective.

ISBN: 978-1-98-856655-9

CHAPTER
18

Depth Studies: Guidance and Ideas

Key terms
citation
depth study
practical investigation
primary data
secondary-sourced investigation
secondary data

Depth study

Key skills and knowledge

Activity number

- ☐ 1 Identify the type of depth study you plan to carry out, e.g. a practical investigation, field study, creation of a simulation or model, data analysis, or secondary-sourced investigation. **220**
- ☐ 2 Spend time planning your depth study to ensure it is successfully completed. Consider whether you have access to the equipment and resources you will need, what type of data you will need to collect and how you will collect and record it, and whether your study can be completed in the required time frame? **220**

Science communication

Key skills and knowledge

- ☐ 3 Use an appropriate communication format to share the findings of your depth study. Whatever format you choose, e.g. scientific poster, essay, practical report, or slide-show, you must use correct scientific terminology, and correct standard abbreviations and units of measurement. **220**
- ☐ 4 Select ways to present your key findings that are appropriate to the design of the study and the type of data. **220**
- ☐ 5 Follow recognised conventions for referencing and acknowledging sources of information. **220**

220 Depth Studies: Guidance and Ideas

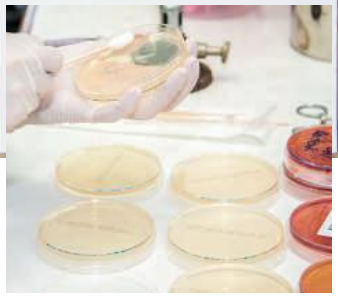
Key Idea: This activity is designed to provide guidance to help you plan and carry out a successful year 12 depth study. A depth study is when you choose an area of the syllabus you have enjoyed or found interesting, and explore it in more depth to deepen your understanding of the topic. In year 12, you will carry out a second depth study. The outcomes are similar to that in year 11. The purpose of this chapter is to provide information to help you plan and carry out a successful depth study. Your teacher will also provide information and guidance to make sure your depth study meets all of the necessary criteria. You can also refer back to chapter 14 in HSC Biology Modules 1-4 for additional information.

Ideas for depth studies

There are many different investigations or activities you could choose for your depth study. Some ideas are provided below. Your depth study may involve a single investigation or activity, or it could be a series of investigations or activities carried out over a longer time frame. You may work alone or in a group with others. Regardless of your approach, as you carry out your depth study you will be developing the Working Scientifically outcomes identified in the NSW stage 6 syllabus, and increasing your knowledge and understanding of the topic.

Practical investigations

- Design and conduct experiments
- Test a claim
- Test a device



Field work

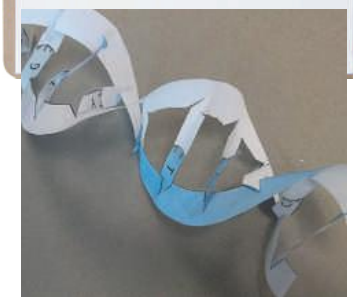
- Design and carry out field work

Data analysis

- Construct and analyse graphs and tables
- Analyse data from a variety of sources
- Analyse research

Creating

- Design and invent
- Create a working model
- Create a portfolio



Secondary-sourced investigations

- Make a documentary or media report
- Conduct a literature review
- Develop an evidence-based argument
- Write a journal article
- Write an essay – historical or theoretical

- Develop an environmental management plan
- Analyse a work of fiction or film for scientific relevance
- Create a visual presentation
- Investigate emerging technologies

ISBN: 978-1-98-856655-9

What is the difference between a practical investigation and a secondary sourced investigation?

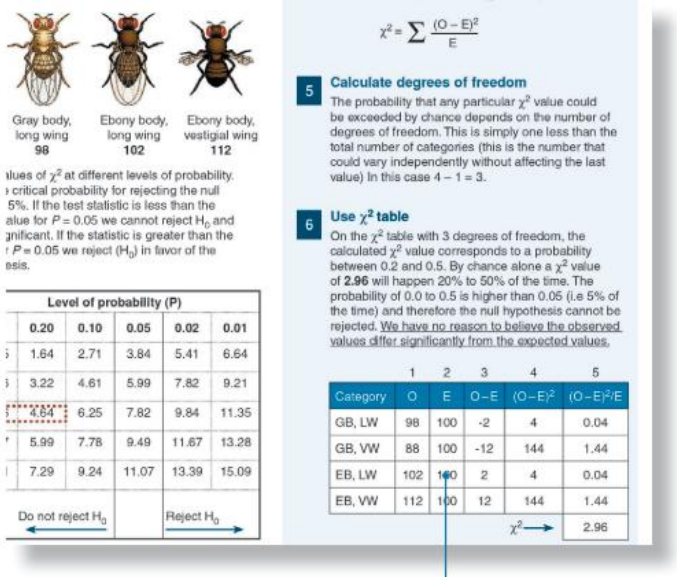
Practical investigation

In a practical investigation, you directly collect data for analysis (primary data). For example, measuring the number of purple or green stems on tomato seedlings, or counting the number of bacterial colonies on an agar plate. Primary data can be gathered through questionnaires and surveys, observations, interviews, and experiments. When planning your investigation it is important to consider what data you will collect, and how you will collect it, so that your analysis can be meaningful. For easy analysis, it is best to collect quantitative data whenever possible.

In this study, a student is carrying out a genetics investigation. They are growing tomato plants to determine the ratio of purple stems to green stems. The data will be recorded in a logbook before analysis. Tables and graphs are an excellent way to present primary data, because trends and patterns in the data can be seen more easily. This student may choose to carry out some basic statistical analysis to determine if the ratios conform to expected outcomes, or if they deviate from expected values.

Secondary-sourced investigation

In a secondary-sourced investigation, you gather, analyse and evaluate other people's data to support or refute a claim. This may include data from studies, surveys, or experiments carried out by other people. You can also gather information from text books or articles. A secondary-sourced investigation may be appropriate when you do not have the equipment or resources to collect primary data yourself. It is also appropriate for gathering together a range of data relating to a specific concept, such as investigating the technical aspects of how a piece of technology works, e.g. a microscope.

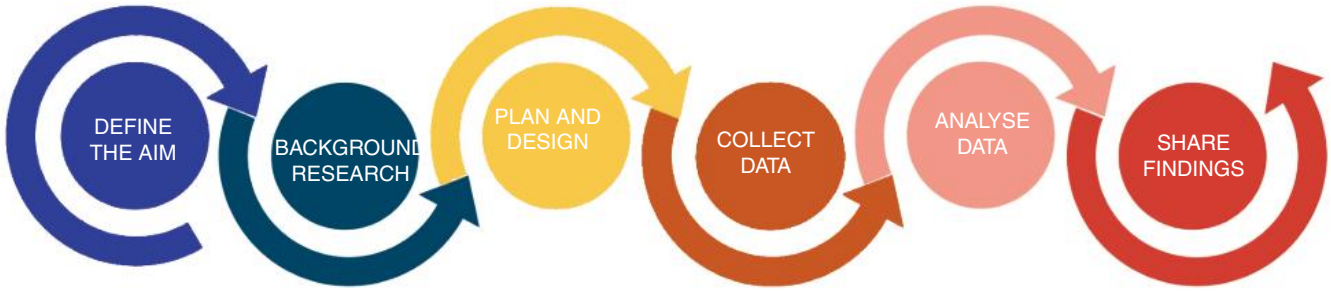


In this book second-hand data is provided

Planning your depth study

- Once you have decided on a topic for investigation, and whether you will carry out either a practical or secondary-sourced investigation, you need to decide what data is required to support your study, and how you will gather it.
- Gathering evidence to test a hypothesis is central to a scientific investigation. For a practical investigation you need to ensure that the methods used to gather and analyse data are fair, without deliberate or unknown bias, otherwise the data may produce results that support flawed hypotheses.
- For example, it is very easy to gather data that supports the idea that light objects fall more slowly than heavy objects. Dropping a feather and a hammer from head height in a closed room will undoubtedly result in the hammer hitting the ground first. However, that is not a valid result because of the biased nature of the test.
- Similarly, gathering secondary data from unreliable sources such as online blog sites, or tabloid magazines and newspapers, is likely to produce bias or flawed results.
- You need to plan how you will organise the data you gather. What statistical tests can be carried out? You may need to carry out preliminary investigations and modify your method, based on the outcome. This can include a literature review to gather background information.
- A simple plan for your depth study is shown below:

How good was the investigation's design? Was it a fair test?



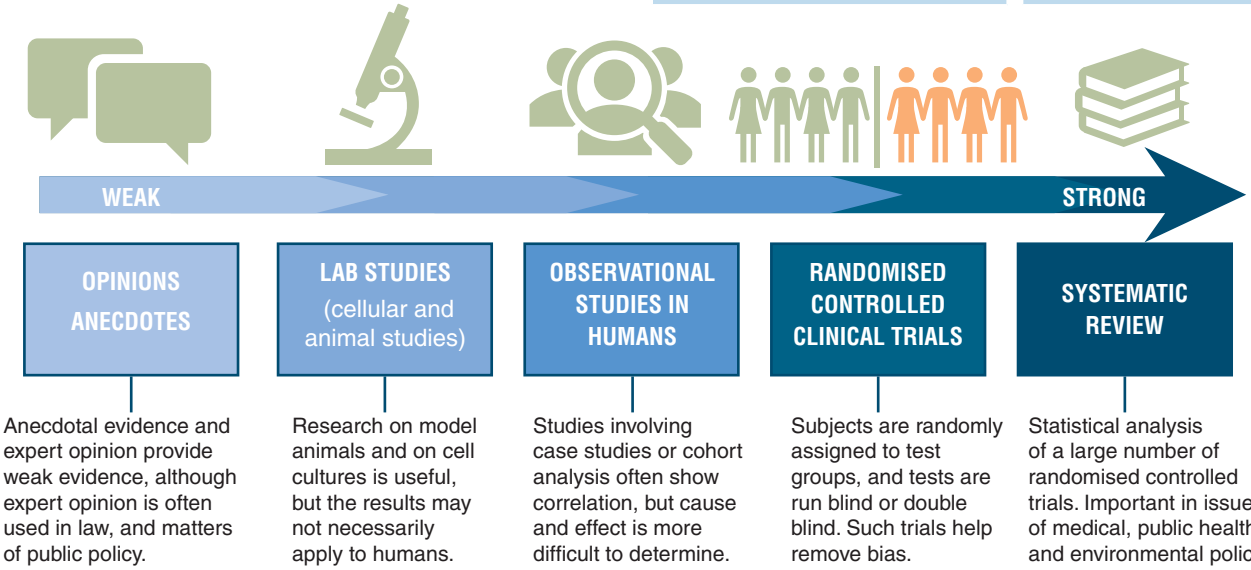
ISBN: 978-1-98-856655-9

In science, it is important that you can critically evaluate and interpret a range of published material in both scientific publications and popular media. To analyse and evaluate the science you read about or see online you must think critically and have a good understanding of the concepts, theories, and models involved.

Opinion, anecdote, and scientific evidence

The validity of scientific claims depends on the evidence used to support the claim. The schematic below shows a hierarchy of evidence that could be used to develop a new pharmaceutical drug. Although this applies to a particular case, the concepts can be applied across all science. Keep this information in mind as you analyse secondary sourced data for your depth study.

ANECDOTAL EVIDENCE	SCIENTIFIC EVIDENCE
Uncontrolled, therefore very susceptible to bias	Controlled for subject and experimenter bias
Very small sample size	Large sample sizes
Only exceptional cases reported	Everything is reported
Vague outcomes	Defined outcomes
Claim from memory	Claim from data



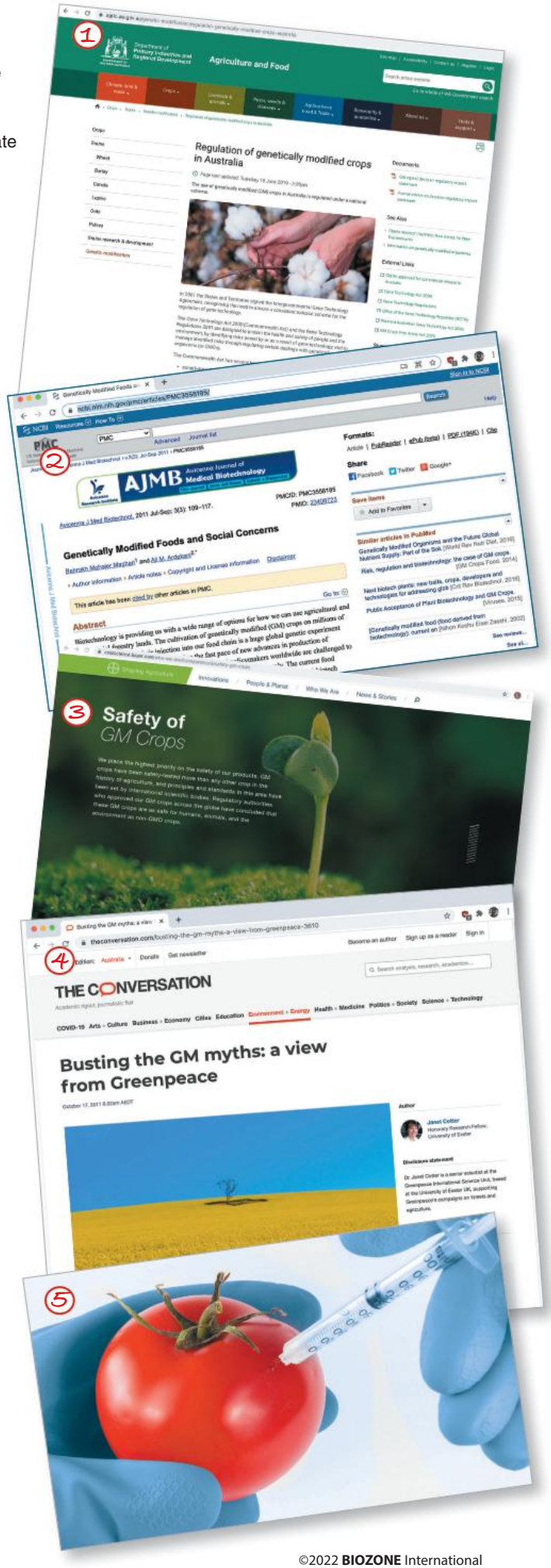
There are many different formats that you can use to communicate and share the findings of your depth study. Some pros and cons of different, common presentation types are given below.

TYPE OF PRESENTATION	ADVANTAGES	DISADVANTAGES
Formal report essay	• Can be used to explain technical concepts, using both words and diagrams. • Paper format makes it simple for handling and reading, and people are often more likely to read printed paper than on-screen notes. • Diagrams and graphs can be displayed easily.	• Generally, the least visually appealing format. • An essay format can be difficult to read and process for some audiences. Font type and size must be chosen carefully, for readability. • The information may be too technical or difficult for some audiences.
Poster presentation	• Presenter not required. • All information is presented on one surface, making access to information simpler. • Can be made visually appealing, producing a good first impression for the viewer.	• Limited size means some supporting figures may need to be reduced in size, or information may need simplifying. • Static images and figures might not always convey the correct idea to viewers.
Slideshow presentation	• Can be viewed with or without a presenter. • Presentation can be brief and contain just the most important facts and figures. • Slide-shows viewed without a presenter provide as much or as little information as required, as there is no physical limit to the file size. • Can be visually appealing to many people and may present information in many ways, e.g. short video clips or animations.	• Presentation software can be tedious to work with and watch, especially if very long or if the information is overcrowded. • It is tempting to add distracting effects, such as words flying in or slides spinning. • Presentations can end up with a large number of slides and become tedious to work through.

ISBN: 978-1-98-856655-9

A guide to referencing online resources

- The internet contains a large amount of information that you may find helpful when carrying out your depth study.
- When you use information from the internet, you must provide the correct details according to the information source (see below).
- The guide below shows how to reference some of the online resources you will commonly find. In all cases, if no publication date is given, replace the date format with (n.d.) for no date.
- Refer back to HSC Biology Modules 1-4 if you need to remember how to reference books and journals.



Referencing Government sites

Website 1 is a government site with no specific author, so the following format would be used:

Organisation name (year, month day). *Page title*. Site name. URL

When the organisation name and page title are the same (yellow highlight) you can remove the page title from your reference.

Government of Western Australia, Department of Primary Industries and Regional Development (2019, June 18). *Regulation of genetically modified crops in Australia*. Government of Western Australia, Department of Primary Industries and Regional Development. https://www.agric.wa.gov.au/genetic-modification/regulation-genetically-modified-crops-australia

Referencing peer reviewed journals

Website 2 is an online journal article with an author. Use this format:

Last name, Initials. (Year). Article title. Journal Name, Volume (Issue), Page range. DOI or URL.

Maghari, B.M & Ardekani, AM. (2011). Genetically modified foods and social concerns. Avicenna J Med Biotechnol., 3(3), pp. 109-117. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3558185/

Referencing company websites

Website 3 is a company website with no author. Use this format:

Organisation name (year, month day). *Page title*. Site name. URL

Bayer (n.d) *Safety of GM crops*. Shaping Agriculture. https://www.cropscience.bayer.com/who-we-are/transparency/a/safety-gm-crops

Referencing online articles

Website 4 is an opinion piece by a Greenpeace scientist NGO's website with an author. Use this format:

Last name, initials. (year, month day). Article title. *Publication name*. URL

Cotter, J. (2011, October 17). Busting the GM myths: a view from Greenpeace. *The Conversation*. https://theconversation.com/busting-the-gm-myths-a-view-from-greenpeace-3610

Referencing social media

Screen 5 represents social media. When referencing social media use this format:

Last name, initials. (year, month day). *First 20 words of post* [description of multimedia aspects] [type of post]. Site name. URL

ISBN: 978-1-98-856655-9

A-1 Appendix 1: Glossary

A

active defence
Defense mechanism that is activated only when a pathogen has been detected.

active immunity
Immunity resulting from the production of antibodies in response to an antigen.

adaptive immune response
The antigen-specific immune response, responsible for immunological memory.

agent (*of disease*)
The non-infectious disease causation, such as tobacco, or alcohol.

Agrobacterium tumefaciens
Species of bacteria commonly used to as a vector to transfer recombinant plasmid DNA to plant cells to produce transgenic plants. The gene transfer is facilitated by the Ti plasmid.

allele
Any of the alternative versions of a gene that may produce distinguishable phenotypes.

allele frequency
The incidence of a particular allele relative to all the alleles of a specific gene.

allergen
A substance causing an immune response in which the immune system overreacts to a normally harmless substance

alpha cells
Endocrine cells found in the pancreatic islets of the pancreas. Synthesise the hormone glucagon.

amino acid
Organic molecule that contains an amino group, carboxylate group, and an R group based on a central C atom. Amino acids are the building blocks of proteins. Twenty amino acids appear in the genetic code.

analytical study
An epidemiological study with a control group, that seeks the how and why of a disease.

anaemia
A condition due to low numbers of oxygen carrying red-blood cells.

aneuploidy
A deviation from the normal number of chromosomes.

angiosperm
Plants that bear flowers and produce seeds enclosed within a fruit. Embryos and endosperm are formed by a double fertilisation.

antagonistic
Hormones that act to ensure the body stays within certain limits by acting against the conditions produced by the other hormone.

antibody
A Y-shaped protein produced by B cells in response to an antigen.

antibiotic
Chemical substance capable of destroying or reducing the growth of microorganisms, especially bacteria and fungi.

anticodon
A sequence of three adjacent nucleotides in tRNA that binds to a corresponding codon in mRNA during protein synthesis (*cf. codon*).

antigen
A molecule that is recognised by the immune system as foreign.

artificially acquired immunity
Any form of immunity produced by deliberate exposure to an antigen (such as a vaccine).

artificial selection
Intervention by humans in animal or plant reproduction in order to preserve selected genetic traits.

asbestos
A naturally found, fibrous mineral used in building construction and insulation. Harmful when inhaled into the lungs.

asbestosis
A disease exclusively caused by inhalation of asbestos fibres, causing damage to alveoli. A risk factor for some types of lung cancer.

asexual reproduction
A type of reproduction involving a single parent, resulting in offspring that are genetically identical to the parent.

autosome
Any chromosome not involved in sex determination.

autosomal dominant trait
A trait or disorder that may be passed from one generation to the next, where only one allele is required to pass on the trait.

autosomal recessive trait
A trait or disorder that may be passed from one generation to the next, where two copies of an abnormal gene must be present in order for the trait to be expressed.

B

B cell
An antibody-producing lymphocyte responsible for humoral immunity.

bacteria
Member of a large group of microorganisms collectively known as prokaryotes. Cells have a cell wall but lack organelles.

beta cells
Endocrine cells found in the pancreas that produce insulin to control blood sugar levels.

binary fission
The process by which one prokaryotic cell divides into two identical daughter cells.

biodiversity
The amount of biological variation present in a region (includes genetic, species, and habitat diversity).

bioinformatics
A subdiscipline of biology and computer science that is concerned with the acquisition, storage, and analysis of biological data.

biosecurity
Procedures and measures put in place to reduce or stop the entrance of unwanted or pest organisms into a country.

budding
A type of asexual reproduction in which a new organism develops from an outgrowth due to cell division at one particular site.

C

carcinogens
Substances and forms of radiation that increase the risk factor for developing cancer.

cardiovascular disease
A general term for any condition that affects the heart and blood vessels, also commonly known as CVD.

cellular immunity
Immunity involving T cells, which attack invading organisms on a cellular level.

cellular pathogen
Pathogen (disease causing agent) that is a living cell. Includes some bacteria, fungi, and protists.

cell therapy
Use of human cells to replace or repair damaged tissue and/or cells. i.e. stem cell treatment.

clone
An individual that is genetically identical to another individual.

Cri du chat syndrome
A rare deletion mutation in chromosome 5 impacting multiple genes, usually the result of a random event

chromosome
A cellular structure consisting of one DNA molecule and associated protein molecules.

chronic
(Of an illness) persisting for a long time.

chronic infection
Infection characterised by the long term or continuing presence of the pathogen and primary infection.

citation
A quotation of, or explicit reference to, a source for substantiation or evidence, as in a scholarly paper.

cohort
A select group in a population under study, normally over time.

clonal selection
An immunological process that determines which B and T lymphocytes will be produced in response to a specific antigen.

cochlea
Structure in ear, containing hair cells that receive vibration from sound waves and transmit electrochemical signals to the auditory cortex of the brain.

cochlear impact
A technological device that replaces lost function of the hair cells and allows a person to hear.

codominance
A phenomenon in which two alleles are expressed to an equal degree within an organism.

codon
A sequence of three adjacent nucleotides in a DNA or mRNA sequence that is part of the genetic code (*cf. anticodon*).

code degeneracy
Property of the genetic code where a single amino acid can be coded for by more than one codon in the genetic code.

coding DNA
DNA that stores the codes for the production of protein in triplets that code for individual animo acids.

colour blindness
Damage to one or more colour cone light receptors that prevents a person 'seeing' the whole range of colours.

comorbidity
A secondary disease that a person has, in addition to another.

control centre
Part of the homeostasis system receiving a message and coordinating a response.

control group
A comparison group in an epidemiological study that does not have the disease or risk factor investigated.

COPD
Chronic obstructive pulmonary disease. Caused by obstructed airflow in the lungs due to inflammation.

countercurrent flow
Occurs when two fluids move past each other in opposite directions, separated by a membrane.

CRISPR-Cas9
A prokraryotic gene sequence used as a tool in the editing of genomes.

crossing over
The reciprocal exchange of genetic material between non-sister chromatids during prophase 1 of meiosis.

cytokinesis
The part of the cell division process when the cytoplasm of a single eukaryotic cell divides to produce two daughter cells.

D

depth study
Investigation that allows for further development of concepts covered in the HSC syllabus.

deletion
Loss of genetic information by the loss of a DNA sequence from the DNA molecule.

dendritic cell
An antigen-presenting cell of the mammalian immune system that processes antigen material and initiates an immune response.

descriptive study
An epidemiological study with no control group, that seeks the what, who, where, and why of a disease.

dialysis
A treatment for kidney function loss that filters waste products from blood using a technological device.

diffusion
Movement of substances from high concentration to low concentration.

dihybrid cross
A cross between two organisms that differ in two observed traits.

diploid
Having paired sets of chromosomes, one from each parent, in a cell or cell nucleus.

disease complex
In a plant, the display of several disease characteristics at once, caused by the invasion of more than one pathogen.

disease prevention
A reduction in risk factors causing disease in order to prevent the disease and/or associated symptoms occurring.

disease transmission
The passing of communicable pathogens between an infected host or group to new potential hosts or group.

DNA (deoxyribonucleic acid)
A large molecule composed of two polynucleotide chains that carries the genetic code and enables cells to function.

DNA ligase
An enzyme that joins DNA strands by catalysing phosphodiester bond formation.

DNA polymerase
An enzyme that catalyses DNA synthesis from nucleoside triphosphates.

DNA profiling
The process of determining an individual's DNA characteristics.

DNA sequencing
A technique used to determine the base sequence (As, Ts, Cs, and Gs) in a DNA molecule.

dominant allele
An allele that is expressed (as a trait) even if the individual only has one copy of the allele.

duplication
Process by which a sequence of DNA is copied and reinserted into the DNA molecule or chromosome.

E

Edwards' syndrome
Also called trisomy 18, the genetically inherited disease is fatal in most babies in the severe form.

effector
In a neural circuit, a muscle or gland that brings about a response.

efficacy
The ability to perform the intended result, specifically, how well a medicine or treatment works.

endoderm
The innermost primary germ layer in a very early human embryo.

endonuclease
An enzyme that cuts the phosphodiester bond within a DNA strand. Endonucleases that cut at specific DNA sequences are termed restriction enzymes.

epigenetics
The study of heritable phenotypic changes that do not involve alterations in the DNA sequence.

epidemic
The rapid spread of disease to a large number of people in a given population within a short period of time.

epidemiology
The study of disease causes, risk-factors, spread, and prevalence.

external validity
A measure of the appropriateness of the case or sample in an epidemiological study, as a representative of a larger group or population.

exon
A protein coding region of a gene.

F

fertilisation
The union of haploid gametes to produce a diploid zygote, initiating the development of a new organism.

fluoridation
Adding fluoride to water supplies, such as drinking water.

fragmentation
A means of asexual reproduction whereby a single parent breaks into parts that regenerate into whole new individuals.

frameshift mutation
A mutation involving the insertion or deletion of a nucleotide(s) in which the number of nucleotides in not divisible by three, causing the triplet code to shift out of sequence.

fungi
Any member of a spore-producing group of eukaryotes that are consumers and have a cell wall made of chitin.

G

gamete
A mature sexual reproductive cell, as a sperm or egg, that unites with another cell to form a new organism.

gametophyte
Haploid multicellular generation in plants with an alternation of generations. The gametophyte produces gametes by mitotic cell division.

gel electrophoresis
The separation and analysis of protein molecules of varying sizes by moving them through a block of gel using an electric field.

gene
A unit of hereditary information consisting of a specific nucleotide sequence in DNA.

genetic cross
The purposeful mating of two individuals resulting in the combination of genetic material in the offspring.

genetic engineering
The techniques associated with the direct manipulation of an organism's DNA or genes in order to alter the outcome of gene expression.

GMO (genetically modified organism)
Any organism whose genetic material has been altered using genetic engineering.

gene drives
Replacement of a natural gene with a modified gene that then gets passed between generations which greater that

normal probability of being passed on, and so quickly propagates through the population.

gene editing
The manipulation of an organism's genetic material by inserting, deleting, or replacing the DNA.

gene therapy
The treatment of disease by altering the underlying genetic condition.

genome
The genetic material of an organism, and all the heritable traits encoded in its DNA.

genetic screening
Technique used to identify individuals who are at higher risk of developing various diseases.

genotype
The genetic makeup of an organism.

genome mapping
Techniques used to identify the location of genes or gene markers on chromosomes.

germination
The development and growth of a seed after a period of dormancy.

germ line
The cells of a multicellular organism that are involved in passing on genes from generation to generation.

glucagon
Hormone that increases blood glucose levels by causing the conversion of glycogen to glucose.

glucose
A simple sugar that functions as the main source of metabolic energy in living things.

glycogen
Multibranched polysaccharide of glucose that stores energy in animals.

goitre
Enlargement of the thyroid gland at the base of the neck, caused by a variety of factors, including by iodine deficiency in the diet.

grafting *(in plant biology)*
Process of joining tissues from two or more sources e.g. joining a stem with roots.

H

hair cells
Receptors within the Organ of Corti in the cochlea that transmit 'sound' to the brain via the auditory nerve.

haploid
Having a single set of each chromosome in a cell or cell nucleus. In most animals, only the gametes are haploid.

haplotype
A set of genetic variations (often SNPs) inherited together on the same chromosome.

herd immunity
A form of indirect protection from infectious disease that occurs when a significant proportion of the population is immune.

heterozygous
Having two different alleles for any hereditary characteristic.

homeostasis
The steady-state physiological condition of the body.

homologous pairs
Pairs of chromosomes, one inherited from each parent, with the same genes in the same order along their chromosomal arms.

homozygous
Having two identical alleles for any hereditary characteristic.

hormone
Chemical messengers secreted directly into the blood, where they circulate to exert specific effects on target tissues and organs.

host
The person with the disease.

humoral immunity
Immunity involving antibodies which circulate through extracellular fluids.

hypermetropia
Short-sightedness, occurring when the eye lens is thickened or the eyeball is elongated.

hypothalamus
The ventral part of the vertebrate forebrain. It functions in maintaining homeostasis, especially in coordinating the endocrine and nervous systems and controlling the secretion of hormones by the pituitary gland.

IJ

infectious disease
A disease caused by a communicable or transmissible pathogen.

immunised (*adjective*)
Having been rendered not susceptible to a disease, through vaccination or exposure.

immunity
Inherited, acquired, or induced resistance to infection by a specific pathogen.

immunological memory
The ability of the immune system to recognise and respond to an antigen that it has previously encountered.

incidence
The rate of new cases occurring in a given population or group over a particular time period.

incomplete dominance
A gene interaction in which both alleles of a gene at a locus are partially expressed, resulting in an intermediate phenotype.

independent assortment
With reference to inheritance, describing how alleles for separate traits are passed to the gametes independently of one another.

infection
The invasion of bodily tissue by pathogenic microbes, their multiplication, and the reaction of host tissues to the pathogens.

innate immune system
Generalised defence mechanisms against pathogens, e.g. physical barriers, secretions, inflammation, and phagocytosis.

insertion
Mutation in which a new DNA sequence is added into the DNA molecule.

insulin
A hormone secreted by pancreatic β cells that lowers blood glucose levels by promoting cellular uptake of glucose, and synthesis and storage of glycogen in the liver.

interferon
Protein released by animal cells in response to infection by a virus. It has the property of inhibiting viral replication.

interleukin-1
Protein involved in the regulation of the immune response, including inflammation and fever.

internal validity
A measure of the strength of the data collection method in an epidemiological study, and how well variables, such as people, place, and time, are controlled.

interphase
The period in the cell cycle when the cell is not dividing, which accounts for about 90% of the cell cycle. During interphase, cellular metabolic activity is high and cell size may increase.

interventional study
An epidemiological study that changes a risk factor or treatment and determines the impact on the disease.

intron
A segment of DNA that does not code for a protein and is removed before translation.

inversion
Mutation that occurs when a piece of DNA in a chromosome breaks, reverses, and is inserted back into the DNA.

***in-vitro* fertilisation**
Laboratory procedure in which egg cells are fertilised outside the body.

KL

Koch's postulates
The criteria that must be met in order to establish the causative relationship between a pathogen and a disease.

LASIK
A medical technology that uses lasers to reshape the cornea to permanently correct vision disorders.

leucocyte (white blood cell)
A type of blood cell that protects against pathogens and can move under its own power. A component of the immune system.

lethal allele
Alleles that cause an organism to die when in the homozygous condition.

linked genes
Genes located on the same chromosome. Functionally refers to genes located close enough together on a chromosome that they tend to be inherited together.

liver
Organ involved in the removal of potential toxins and synthesis of proteins and hormones and carbohydrate homeostasis.

locus (*pl., loci*)
A specific place along the length of a chromosome where a given gene is located.

longitudinal study
A study following a group, or cohort, over time.

lymph
A clear fluid, containing white blood cells, that flows through the lymphatic system.

lymph node
Small masses of lymphoid tissue that filter bacteria and foreign particles from lymph.

lymphocyte
T and B white blood cells responsible for the adaptive immune response.

M

macroorganism
An organism large enough to be seen with the unaided eye.

macrophage
A large phagocytic leucocyte, which engulfs and devours invading cells.

major histocompatibility complex
A tightly linked cluster of genes that code for cell surface proteins essential for the adaptive immune system.

marker gene
A gene with a known location used to track the insertion of DNA into organisms.

meiosis
The process of double nuclear division in sexually reproducing organisms, which results in cells with half the original number of chromosomes (haploid).

Mendelian inheritance
Patterns of inheritance that are characteristic of organisms that reproduce sexually, such as independent assortment and segregation of chromosomes.

mesothelioma
A rare cancer of the pleura (lung lining) that is fatal, and most often caused by inhalation of asbestos fibres.

microorganism
An organism small enough that it can not be seen with the unaided eye, i.e. tools such as microscopes are required.

micropropagation
The process of cloning plants through tissue culture or cell culture techniques.

microsatellite
A segment of DNA consisting of multiple repetitions of short sequences of base pairs.

mitosis
The phase of the cell cycle resulting in nuclear division.

missense mutation
A single nucleotide mutation, coded for by the DNA sequence, that results in an incorrect animo acid.

monohybrid cross
A cross between two organisms that differ in one observed trait.

morbidity
A measure of those afflicted from a disease.

mortality
A measure of the number of deaths in a population per unit of population, per unit of time.

mutagen
Anything that causes a mutation in the DNA code, including chemicals, and UV radiation.

multiple alleles
The existence of more than two alleles for a gene in the population.

multiregional hypothesis
Theory that modern humans evolved by multiregional evolution with continuous gene flow between groups throughout the Old World.

myopia
Long-sightedness, occurring when the eye lens is too thin or the eyeball is shortened.

mutation
A change in the nucleotide sequence of an organism's DNA (or RNA).

N

nanomedicine
The use of ultra-small 'nano' materials and technology to treat disease.

naturally acquired immunity
Immunity developed through exposure to an infectious agent or other antigen.

negative feedback
In physiology, a primary mechanism of homeostasis where a change in a variable triggers a response that counteracts the initial change.

nephron
The functional unit of the kidney, consisting of the glomerulus and associated tubules which filter the blood and produce urine.

noncellular pathogen
A pathogen that is not a living cell. Includes viruses and prions.

noncoding DNA
DNA sequence that does not code for the amino acids in proteins.

Non-infectious disease
Disease that is not transmissible, with the exception of inheritance, and is often caused by genetic or lifestyle factors. Also referred to as non-communicable disease (NCD).

nonself antigen
Antigens that do not originate in an organism's own body.

nonsense mutation
A mutation in the DNA that causes a termination triplet to appear prematurely in the DNA code and causes the production of the protein to be prematurely terminated.

nucleic acid
A polymer (poly-nucleotide) consisting of many nucleotide monomers; serves as a blueprint for proteins and, through the actions of proteins, for all cellular activities. The two types are DNA and RNA.

nucleotide (pl., nucleotides)
An organic molecule that is the building block of DNA and RNA.

neuron
Cell of the nervous system that transmits information to other nerve cells.

O

observational study
A type of epidemiological study that uses data, rather than interacting with the disease progression of patients.

oestrogen
One of the main female sex hormones. It is responsible for the development of the reproductive system in and secondary sex characteristics of females

oogenesis
The process in the female reproductive system in which the primary oocyte matures in the ovum.

osmosis
The diffusion of free water across a selectively permeable membrane.

osteoporosis
Nutritional disease that reduces the density and strength of bones, often resulting in fractures. Risk factors include lack of calcium in the diet, old age; more common in women.

outbreak *(disease)*
A sudden increase in the occurrence of a disease in a particular time and place.

Out-of-Africa hypothesis
Theory that modern humans evolved in Africa and then migrated across the globe, replacing other *Homo* species.

ovum
Female reproductive cell (gamete). Also called the egg cell.

P

pancreas
Organ that produces enzymes that aid digestion and hormones that regulate blood glucose levels (insulin and glucagon).

pandemic
A disease that is continuing to spread and which has become prevalent throughout most of the world.

passive defence
Defenses that are always present and that are not the result of contact with a pathogen or grazer (in plants).

passive immunity
Immunity acquired by the transfer of antibodies from another individual.

Pasteur
Louis Pasteur. French chemist and microbiologist who founded many principles of modern microbiology, disproving spontaneous generation, developing the germ theory of diseases, and the process of pasteurisation.

pathogen
Microorganism that causes disease.

Patau syndrome
Also called trisomy 13. This chromosome mutation arises during meiosis and causes restricted growth of the fetus in the uterus, with the baby usually having low birth weight and severe heart defects.

phagocyte
A cell that protects the body by engulfing and ingesting harmful foreign particles, bacteria, and dead or dying cells.

pedigree
A diagram of a family tree with conventional symbols, showing the occurrence of heritable characters in parents and offspring over multiple generations.

period prevalence
The total burden of a specified disease during a specified period of time.

phenotype
The observable physical, behavioural, and physiological traits of an organism.

phenylketonuria (PKU)
Genetic disease resulting in the inability to metabolise the amino acid, phenylalanine.

pituitary gland
Endocrine gland situated at the base of the brain, often referred to as the master gland as it controls the functioning of most other endocrine glands.

plasmid
A circular, extra-chromosomal segment of DNA, capable of self replication.

pollination
In plants, the process of transferring pollen from the anthers (male) to the stigma (female). May be achieved by moving pollen by wind, insects, or water.

point prevalence
The total burden of a specified disease at a given time.

polymerase chain reaction (PCR)
An *in-vitro* technique for rapid synthesis of a given DNA sequence.

polypeptide
A chain of amino acids linked together by peptide bonds.

polyploidy
A condition in which the cells of an organism have more than two complete sets of chromosomes.

practical investigation
Activity that involves the collection of data for processing and interpretation by manipulating specific variables in real or modelled situations.

prevalence
The proportion of the population afflicted with a specific disease at any given time point or period.

primary data
Data collected from first-hand sources.

primary response
The response displayed by the immune system when first exposed to an antigen.

primary structure
The linear sequence of amino acids in a polypeptide chain.

prion
Abnormally folded pathogenic protein that can induce other similarly structured proteins to also fold abnormally.

probability
The chance or likelihood that a certain event will occur or that a prediction will be correct.

protein
A biologically functional molecule consisting of one or more polypeptides folded into a specific three-dimensional structure.

protist
Single cell organisms belonging to the eukaryotic kingdom Protista.

Punnett square
A diagram used in the study of inheritance to show the predicted genotypic results of random fertilisation in genetic crosses between individuals of known genotype.

Q

quarantine
Disease control mechanism to isolate exposed individuals that *may* be infected.

quaternary structure
The structure formed by the association of two or more polypeptides.

qualitative data
Non-numerical data that describes qualities or characteristics.

quantitative data
Numerical data expressing a certain quantity, amount, or range.

R

receptor
A protein molecule inside a target cell or on its surface that receives a chemical signal and brings about a response.

recessive allele
An allele that is only expressed if the individual has two copies of the allele (also known as being homozygous).

recombination
The process by which genes are exchanged between different chromosomes to produce new combinations of alleles.

recombinant DNA
Genetically engineered DNA made by recombining DNA from different organisms.

reproductive technologies
Laboratory techniques used to manipulate and enhance the reproductive process. Includes IVF and PGD.

response
Any behaviour of a living organism that results from an external or internal stimulus.

reverse transcriptase
DNA polymerase enzyme that transcribes single stranded RNA into DNA.

retina
Lining of the inside of the eyeball that contains the rod and cone light receptors.

rickets
Nutritional disease that causes deformed leg bones in children. Caused by Vitamin D deficiency.

RNA (mRNA, rRNA, tRNA)
A long (generally) single-stranded nucleic acid, essential in various biological roles including gene expression.

RNAi
RNA interference. A molecular mechanism in eukaryotes that targets and cleaves specific mRNA molecules and stops them from being translated.

S

seed
The unit of reproduction in plants. Produced by fertilisation of the egg cell by the sperm cell. Capable of growing into an independent plant.

secondary response
Immune response that occurs as a result of the second (and subsequent) exposure to a particular antigen.

secondary data
Pre-existing data that has already been collected from first-hand sources.

secondary sourced investigation
Investigation using that analyses and evaluates secondary sourced information.

self antigen
Antigen produced in the organism's own body and is recognised as such by the immune system.

semi-conservative replication
The normal mechanism of DNA replication, where each strand acts as a template for a new double helix.

sex chromosome
Chromosomes involved in sex determination.

sex linkage
An association between genes in sex chromosomes that makes some characteristics appear more frequently in one sex.

sexual reproduction
A type of reproduction in which offspring are created by combining genetic information from two individuals of different sexes.

silent mutation
Mutations that does not have an observable effect on an organism's phenotype.

single nucleotide polymorphism (SNP)
Single nucleotide change in a DNA sequence that is present in at least 1% of the population.

somatic
Cells that are of the body, rather than reproductive cells.

somatic cell nuclear transfer
(SCNT) Cloning technique in which the nucleus of an animal's somatic cell is transferred into the enucleated egg cell of a donor animal of the same species. The process produces a genetically identical clone of the original animal.

sperm
The male reproductive cell (gamete). In animals, the cell is motile.

spermatogenesis
The process in the male reproductive system by which mature sperm cells are produced.

spinal muscular atrophy
A rare genetic disorder that causes progressive destruction to motor neurons due to a mutation in a vital 'repair' protein gene (SMN1).

Spinraza®
An SMA gene therapy that delivers synthetic nucleotides directly into the cells to induce repair proteins by a similar gene (SMN 2) to compensate for the mutated SMN1 gene.

spore
A reproductive cell capable of asexual reproduction, adapted for dispersal and for survival in unfavourable conditions.

sporophyte
The diploid multicellular stage of the plant life cycle. The sporophyte produces spores by meiosis, which develop into the gametophyte.

stimulus
In neural or hormonal circuits, a fluctuation in a variable that triggers a response.

stoma (plural, stomata)
A microscopic pore, found in the epidermis of leaves and stems, that allows gas exchange between the plant and the environment.

substitution
Mutation in which a nucleotide(s) is replaced with a different nucleotide(s) in the DNA.

T

T cell
A type of lymphocyte that plays a central role in the adaptive immune response.

T killer cell
A type of T lymphocyte that kills infected cells, cancer cells, and damaged cells.

T helper cell
A type of T lymphocyte that recognises foreign antigens and releases cytokines that activate T and B cells.

Tay-Sachs disease
Rare genetic disorder, caused by a mutation in the HEXA gene on chromosome 15, that results in the inability to produce enzymes that breakdown fats.

tertiary structure
The three-dimensional structure of a protein, created by the folding of the helices or sheets.

testosterone
The male reproduction hormone. Responsible for the development of the male reproductive system and secondary sexual characteristics.

thermoreceptor
Sense receptor that responses to and transmits information about relative changes in temperature.

thermoregulation
The maintenance of internal body temperature within a tolerable range.

thyroid gland
Endocrine gland location in the neck, lying across the trachea. Secretes hormones that regulate the body's metabolic rate.

thymus
A primary lymphoid organ of the immune system, where T cells mature.

trait
One of two or more detectable variants in a genetic character.

transcription
The process of copying a segment of DNA into a strand of mRNA.

transgene
A gene which is artificially transferred from one organism to another.

transgenic
An organism in which the genome has been altered by the insertion of a novel gene.

translation
The process of decoding a strand of mRNA to produce a sequence of amino acids.

translocation
Mutation in which part of the DNA or chromosome is shifted from one area of the DNA molecule or chromosome to another.

transpiration
The evaporative loss of water from a plant.

type 1 diabetes
Auto-immune disease in which the insulin-making cells are destroyed by the immune system.

type 2 diabetes
A non-infectious disease caused by the inability of cells to respond adequately to normal levels of insulin.

UVW

water potential
The potential energy of water in a certain environment, compared with the potential energy of pure water at room temperature and atmospheric pressure.

vaccination
Inoculation with a vaccine to help the immune system develop immunity to a particular disease.

vaccine
A substance used to stimulate the production of antibodies and provide immunity against specific disease.

validity
The quality and accuracy of the research or study.

vector
Any living organism that carries and transmits a disease-causing pathogen between hosts.

virus
Non cellular pathogen consisting of genetic material (DNA or RNA) surrounded by a protein coat and which is unable to reproduce without infecting a cellular host.

XYZ

xenotransplantation
Transplantation of an organ or tissue from one species to another, often experimental.

X-linkage
Sex linkage involving the X chromosome.

Zolgensma®
An SMA gene therapy that introduces a new working copy of the SMN1 gene into the body, using a viral vector.

zygote
A fertilised egg.

A-2 Appendix 2: Equipment List

The equipment list provides the material and equipment needed per student, pair, or group.

1: Reproduction

INVESTIGATION 1.1
Plant propagation

Per student/pair
9 x plant/seed containers or trays
3 x planting mediums (e.g. sand, bark, potting mix)
Rooting hormone
9 x ice block sticks
Secateurs or scissors
Measuring flask or container for water

INVESTIGATION 1.2
Germination investigation

4 x plant/seed trays
4 x sets of 100 tomato seeds or similar (e.g. mustard seeds)
Sterilised growing medium
Measuring flask or container for water

2: Cell Replication

INVESTIGATION 2.1
Modelling mitosis

String
4 x pipe-cleaners (2 colors) cut in half
A3 sheet of paper
Marker

INVESTIGATION 2.2
Modelling meiosis using ice block sticks

Per pair
16 x ice block sticks
Sticky dots
Marker pen

INVESTIGATION 2.3
Extracting DNA

Per pair
5 - 6 strawberries
1 large zip-lock bag
100 mL water
5 mL detergent
pinch of salt
1 x filter paper
1 x glass filter funnel
1 x 250 mL glass beaker
1 x glass rod
~100 mL ethanol (for rinsing)
2 x centrifuge tubes
Centrifuge

INVESTIGATION 2.4
Creating a model of a DNA molecule

Per pair
Scissors
Tape or paste

3: DNA and Polypeptide Synthesis

INVESTIGATION 3.1
Measuring continuous variation

Per pair
Measuring tape or scales
Graph paper

INVESTIGATION 3.2
Separating amino acids

Per student/pair
Filter paper or chromatography paper
Pencil
Clingwrap or parafilm
Scissors
1% amino acid solutions (leucine, lysine, glycine).
Chromatography solution (butan-2-ol, glacial ethanoic acid, water in ratio 6:1.5:2)
Ninhydrin spray or black light
Nitrile gloves

INVESTIGATION 3.3
Modelling protein structure

Per student/pair/group
Pipe cleaners (2 white, 2 pink, 2 purple, 4 blue)
Sticky tape
2 x binder clips or paper clips

4: Genetic Variation

INVESTIGATION 4.1
Measuring continuous variation

Computer with spreadsheeting programme e.g. Excel.

6: Mutation

INVESTIGATION 6.1
Investigating natural selection

Per student
Computer
Spreadsheet application (e.g. Excel)

INVESTIGATION 6.2
Modelling genetic drift

Per student
Computer
Spreadsheet application (e.g. Excel)

9: Causes of Infectious Disease

INVESTIGATION 9.1
Investigating microbial contamination in food samples

Per student or group
Food sample
Agar plates
Inoculation loops
Bunsen burner
Sterilising alcohol
Test tubes
Glass rods
Distilled water
Tape
Marker pens
Incubator

12: Prevention, Treatment and Control

INVESTIGATION 12.1
Investigating the effectiveness of handwashing

Per class
Warm water
Soap
Hand sanitiser

Per individual
1 x nutrient agar plates
Marker pen
Paper towels
Incubator (if using)

INVESTIGATION 12.2
Modelling disease outbreak and spread

Per pair
Computer
Spreadsheet application (e.g. Excel)

Credits

ACKNOWLEDGEMENTS
The writing team would like to thank the following people and organisations for their contributions to this edition:• Dr David Wells, AgResearch for the image of the cloned embryos • Cytogenetics Department, Waikato Hospital, Hamilton, NZ for the images and information on chromosomal abnormalities and karyograms • Dr David Wells, AgResearch for the images of the cloned calves • Scott McDougall for the photo of the monarch butterfly caterpillars

SPECIAL REFERENCES

Source material for genetic gain in diary animals (page 46): T.S. Sonstegard *et al* (2008). Genomic signatures of artificial selection in U.S. Holstein cows. ISAG Proc. Poster 2098. ars.usda.gov

Source image used for Tasmanian devil MHC analysis (page 165): Cheng et al.: Antigen-presenting genes and genomic copy number variations in the Tasmanian devil MHC. BMC Genomics2012 13:87. cc 2.0

Source material used for Tasmanian devil MHC analysis (page 165): MHC diversity and female age underpin reproductive success in an Australian icon; the Tasmanian devil Tracey Russell et al www.nature.com, 8 March 2018 CC 4.0

Source material used for breast cancer gene data (page 170): Chunling Hu et al, (2021). A population-based study of genes previously implicated in breast cancer. The New England Journal of Medicine 384:440-451. DOI: 10.1056/NEJMoa2005936

Source material used for human genetics data (page 171): The 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature 526, 68–74 (2015). https://doi.org/10.1038/nature15393

Source material for the populating of Australia (page 172): R. Tobler *et al* (2017). Aboriginal mitogenomes reveal 50,000 years of regionalism in Australia. Nature 544. doi:10.1038/nature21416

Source data for the percentage of Neanderthal or Denisovan DNA in modern human groups (page 173): Sankararaman, S., Mallick, S., Dannemann, M. et al. The genomic landscape of Neanderthal ancestry in present-day humans. Nature 507, 354–357 (2014). https://doi.org/10.1038/nature12961

Source data for genetic diversity in playtpus populations (page 203): Mette Lillie et al (2012). Diversity at the Major Histocompatibility Complex Class II in he Platypus, Ornithorhynchus anatinus. Journal of Heredity 103(4), 467–478.

Source paper for protein content in maize (page 224) Moose SP, Dudley JW, Rocheford TR. Maize selection passes the century mark: a unique resource for 21st century genomics. Trends Plant Sci. 2004 Jul;9(7):358-64. doi: 10.1016/j.tplants.2004.05.005. PMID: 15231281.

Source paper for plot of artificial selection in *B. rapa* (page 224) Zu, P. and Schiestl, F.P. (2017). The effects of becoming taller: direct and pleiotropic effects of artificial selection on plant height in *Brassica rapa*. Plant J, 89: 1009-1019. https://doi.org/10.1111/tpj.13440

Source data for effect of myrtle rust on rainforest structure (page 284): Pegg G, Taylor T, Entwistle P, Guymer G, Giblin F, Carnegie A (2017) Impact of Austropuccinia psidii (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland. PLoS ONE 12(11): e0188058. https://doi.org/10.1371/journal.pone.0188058

Source data for data showing response on Australian trees to infection by *Phytophthora cinnamomi* (page 290): K. H. Kueh,S. F. McKay,E. Facelli,J. M. Facelli,R. M. A. Velzeboer,A. J. Able,E. S. Scott (2012). Response of selected South Australian native plant species to Phytophthora cinnamomi. Plant Pathology 61(6). https://doi.org/10.1111/j.1365-3059.2012.02593.x

Source data for Influenza deaths in Australia (page 303): Compiled from Australian Institute of Health and Welfar (https://www.aihw.gov.au/reports/burden-of-diease/abds-2015-interactive-data-disease-burden/contents/burden-of-disease-in-australia)

Source data for the effectiveness of handwashing (page 311): Burton M, Cobb E, Donachie P, Judah G, Curtis V, Schmidt WP. The effect of handwashing with water or soap on bacterial contamination of hands. Int J Environ Res Public Health. 2011 Jan;8(1):97-104. doi: 10.3390/ijerph8010097. Epub 2011 Jan 6. PMID: 21318017; PMCID: PMC3037063.

Data for Covid-19 notifications in Australia (page 331)
Australian Government Department of Health (14 March 2021). Covid-19 Australia: Epidemiology Report 37. Retrived from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/1D03BCB527F40C8BCA258503000302EB/

Source data for handwashing techniques (page 333): Maliekal, M., Hemvani, N., Ukande, U., Geed, S., Bhattacherjee, M., George, J., & Chitnis, D. S. (2005). Comparison of traditional hand wash with alcoholic hand rub in ICU setup. Indian J Crit Care Med. Vol 9(3).

Source data for infectious vs non-infectious disease mortality (page 354): Armstrong GL, Conn LA, Pinner RW. Trends in infectious disease mortality in the United States during the 20th century. JAMA. 1999 Jan 6;281(1):61-6. doi: 10.1001/jama.281.1.61. PMID: 9892452.

Source data for cancer data by type (page 357): Wilson LF, Antonsson A, Green AC, Jordan SJ, Kendall BJ, Nagle CM, Neale RE, Olsen CM, Webb PM, Whiteman DC. How many cancer cases and deaths are potentially preventable? Estimates for Australia in 2013. Int J Cancer. 2018 Feb 15;142(4):691-701. doi: 10.1002/ijc.31088. Epub 2017 Oct 26. PMID: 28983918.

Source data for obesity in children (page 360): ABS 2019a. Microdata: National Health Survey, 2017–18. ABS cat. no. 4324.0.55.001. Canberra: ABS. Customised data report.

Source data for COPD and smoking deaths (page 361): Australian Institute of Health and Welfare, Poulos LM, Cooper SJ, Ampon R, Reddel HK and MarksGB 2014. Mortality from asthma and COPD in Australia. Cat. no. ACM 30. Canberra: AIHW.

Source material for self reported Type 2 diabetes data (page 364): Australian Institute of Health and Welfare (2019). Indicators of socioeconomic inequalities in cardiovascular disease, diabetes and chronic kidney disease. DOI: Link to DOI:10.25816/5ebca9d1fa7e0

Source material for socioeconomic and risk factors on CVD and type 2 diabetes in Australia (page 369): Li J, Kinfu YImpact of socioeconomic and risk factors on cardiovascular disease and type II diabetes in Australia: comparison of results from longitudinal and cross-sectional designs BMJ Open 2016;6:e010215. doi: 10.1136/bmjopen-2015-010215

Source for dental health data in Australian children (page 370): Ha DH, Roberts-Thomson KF, Arrow P, Peres KG & Spencer AJ 2016. Children's oral health status in Australia, 2012–14. In: Do LG & Spencer AJ (eds). Oral health of Australian children: the National Child Oral Health Study 2012–14. Adelaide: University of Adelaide Press.

Source for tobacco use in Australia (page 375): ABS (2019) Customized report: National Health Survey 1989-90, 1995, 2001, 2004-05, 2007-08, 2014-15, 2017-18; Australian Health Survey.

PHOTO CREDITS

We acknowledge the photographers who have made images available through Wikimedia Commons under Creative Commons Licences 0, 1.0, 2.0, 2.5, 3.0, or 4.0:
• Curtis Clark • Kalyan Varma • Tim Evanson • Melburnian • Sabina Bajracharya • J. Arthur Thomson • Ernie • Neutr0nics • Christian Fischer • Ernest F • Sander van der Wel • Bob Embleton • Zwifree • Brocken Inaglory • Bill Rhodes • Jpbarrass • Ed Uthman • dsworth Center- New York State Department of Health • Yaminchhipa10 • Fdardel • emNL • Bolzer et al PLOS (2005) • Jee & Rani Nature Photography • Noor et al PLOS • msdonna • Cindy McCravey • Ericlin1337 • Christian Schmelzer • Jakob Suckale • SubtleGuest • Bone Clones • Palewhalegail • Emmanuelm • Bill Ebbesen • Diacritica • Soviet Authorities Fair Use • Dr Graham Beards • NYWTS • Agnieszka Kwiecie • Honeyhuyue • Giorgiogp2 • Klaus • Nzeemin • Tentotwo • CDC: Janice Haney Carr • Fred Brooks, University of Hawaii • Forrest Brem • Ute Frevert, Plos • Edward L. Barnard, Florida Department of Agriculture and Consumer Services, Bugwood.org • John H. Ghent, USDA Forest Service, Bugwood.org • Jpatokal • CDC: Tara Sealy • Rasbak • Scott Bauer USDA • DOC. RNDr. Josef Reischig • CDC: Rob Weyant • Bjørn Christian Tørrissen • DRdoubleB • Geoff Derrin • lookscloser • John Tann • Ccroberts • Taro Taylor • Obli • Volker Brinkmann PLOS • EMW GS •Bjørn Christian Tørrissen • Mark Marathon • John Jennings • Geoff Derrin • Casliber • Ragesoss • Ildar Sagdejev • RM Hunt • Toni Barros • 25kartika • Romain Behar • Dual Freq • IRRI • David R. Tribble • Greenpeace • James Heilman, MD • Wellcome Collection. Attribution 4.0 International • Hear hear! • John Doebley • www.glofish.com • Scott Bauer, USDA ARS • Melburnian • Grant Singleton CSIRO • The Wednesday Island Thomas Schoch

Contributors identified by coded credits are:

• CDC: Centers for Disease Control and Prevention, Atlanta, USA •DH: Don Horne • EII: Educational Interactive Imaging • KP: Kent Pryor • NASA: National Aeronautics and Space Administration • NCI: National Cancer Institute • NIAD: National Institute of Allergy and Infectious Diseases • NIH: National Institute of Health • USAF: United States Air Force • USDA: United States Department of Agriculture • USDE: United States Department of Energy • WMU: Waikato Microscope Unit

Royalty free images, purchased by BIOZONE International Ltd, are used throughout this workbook and have been obtained from the following sources: • Adobe Stock • iStock images • Corel Corporation from their Professional Photos CD-ROM collection; ©Digital Vision; PhotoDisc®, Inc. USA, www.photodisc.com • Science Photo Library • 3D images created using Bryce, Poser, and Pymol • https://unsplash.com/

Index