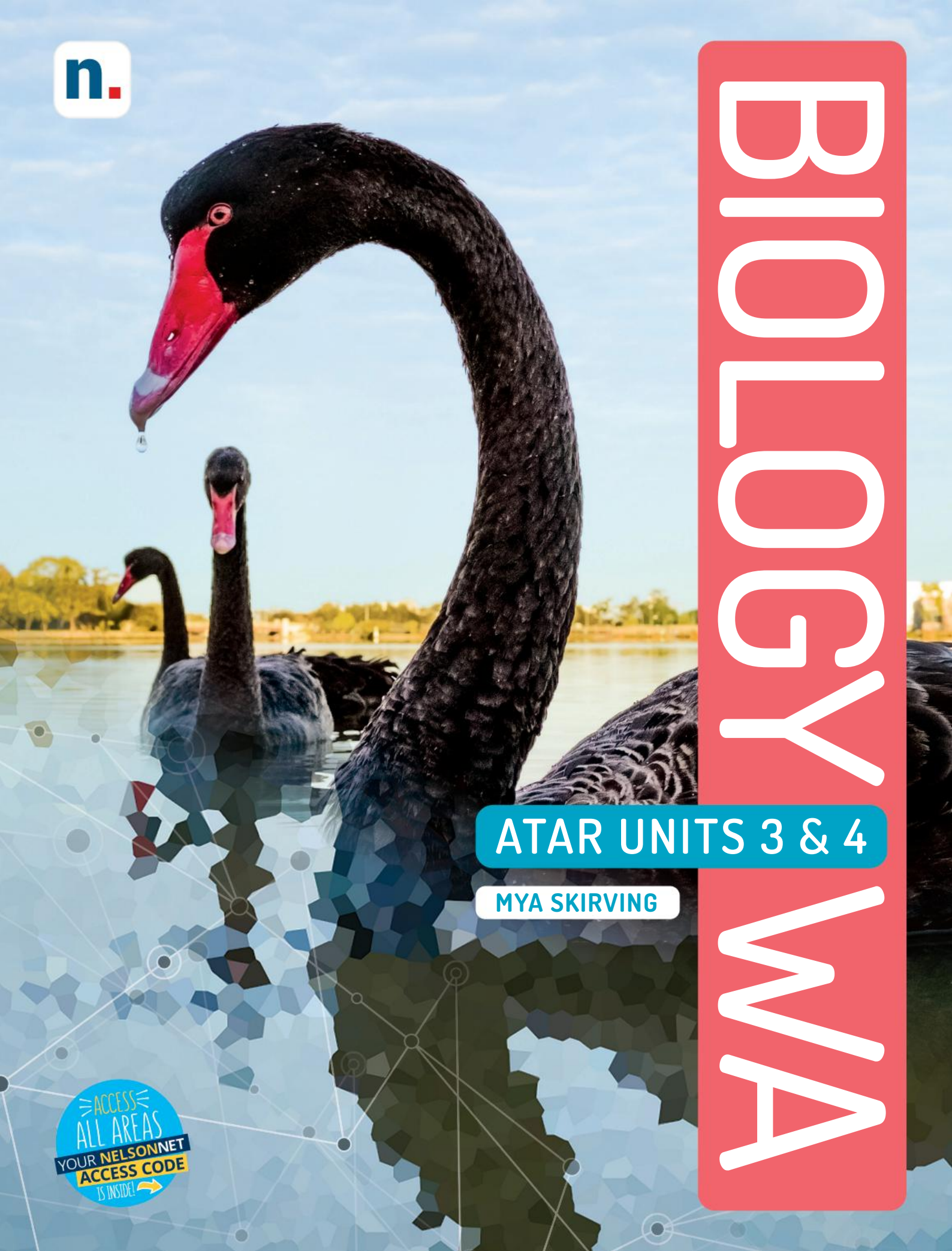

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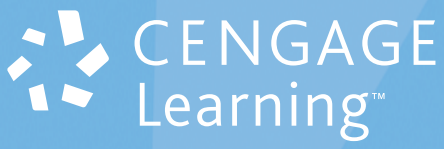

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ATAR UNITS 3 & 4

MYA SKIRVING

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FOREWORD BY LYN BEAZLEY

I am delighted and honoured to provide a preamble for this very special book.

I have been inspired by biology since my youngest days back in England, spurred on by a school visit to Charles Darwin's house and a chance to look through his microscope. I was then off to Oxford and Edinburgh to study biology, but it was only when I moved to Western Australia that I realised just how extraordinary the life forms on our planet truly are.

I came from a part of the world that has around 1500 species of flowering plants. Western Australia, a world-recognised biodiversity hotspot, has many times that number, spanning diminutive subterranean orchids to among the tallest trees in the world. Then there are our amazing land animals – aestivating frogs, marsupial moles, echidnas, honey possums (feeding exclusively on pollen and nectar), numbats and kangaroos. The creatures on our coasts and in our oceans range from elegant sea dragons and fairy penguins to mysterious whale sharks, the largest fish in the world.

Our amazing biodiversity exists across a vast land mass that varies from tropical to temperate, and is very occasionally icy. It has both lush forests and deserts, and is framed by reef-fringed coasts and deep oceans. Australia has been shaped in isolation over millions of years as it drifted north from the supercontinent Gondwanaland. It has been less impacted by the disruptive effects of glaciation and volcanic activity than many parts of our planet. Yet now we are witnessing the impact of climate change within a human lifetime.

I can think of no better way to introduce biology to our school students than through a book such as this, based on Western Australian examples of animals and plants with which they have grown up.

I hope the next generation of our students enjoy and benefit from this book, learn from it and are inspired, so they in turn can play a part in ensuring this most precious part of planet Earth is protected and can sustain generations to come.

Lyn Beazley AO, FAA, FTSE

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Author
Mya Skirving

During Mya Skirving's teaching career, she has completed a Master of Science Education degree and been appointed as Science Curriculum Coordinator, WACE and HSC Biology Marker (over a 12-year period), Level 3 Classroom Teacher, and 2iC in the science department of an academic selective school. Several of her students have been awarded a WACE Subject Exhibition. She enjoys providing useful resources for other teachers as well as students, and worked for a number of years with university students as a Teacher Advocate. Her love of the biological world, coupled with her love of education, inspired her to write this set of textbooks and NelsonNet material to fill the need for a biology resource with a particularly Western Australian flavour.

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Reviewer
Jane Brandenburg
Jane fills a number of roles at a top-ranked Western Australian school, including Learning Coordinator, Creativity Activity Service Coordinator and Year Coordinator. She is also a teacher of Years 11 and 12 Biology and Human Biology, and of Science and Mathematics, including the International Baccalaureate Middle Years Programme.

Consultant
Helen Lydon
Helen is a key advocate for and influencer of biology education in Western Australia. She is also an Associate Principal at the Department of Education and Training, Western Australia, and a practising Association of Independent Schools of Western Australia teacher of Year 12 Biology.

AUTHOR'S ACKNOWLEDGEMENTS

I am sincerely appreciative of my very supportive and reassuring husband (David), our three kids (Saasha, Sophie and Justin) and our dog (Leo). Their random acts of kindness (cooking and coffees, Reabold Hill and riding), endless patience waiting for me to finish writing the various sections, and hugs eased any stress and made this experience achievable and enjoyable. Thank you – you all bring sunshine into my life.

The students, colleagues and scientists I have worked with, past and present, have all taught and inspired me, especially Ant, who had faith in my ability.

I am in awe of the beauty and resilience of our natural biological world. Nothing humans have created even comes close. I hope this book plays a part in preserving it, and I hope it will inspire passionate science students to solve big biological problems, such as infectious disease control, wise biotechnology advancement, and ecosystem rejuvenation.

Special thanks go to the following scientists, who even when busy made time for me, vastly improving the integrity of the material presented here: Lyn Beazley, extraordinary former chief scientist of WA and inspiration for many of us; Pauline Charman, former Education Outreach Manager, Harry Perkins Institute of Medical Research, a science educator with a unique skill set and knowledge in the area of biotechnology; Stephen D Hopper AC, Professor of Biodiversity, Centre of Excellence in Natural Resource Management, School of Agriculture & Environment, UWA; Professor John S Mackenzie, Emeritus Professor, Curtin University, Co-initiator and Vice-chair of One Health, world expert in Ross River virus and Australian bat lyssavirus, and Consultant for the WA Biosecurity Council and WHO (Steering Committee of the Global Outbreak Alert and Response Network); Dr Peter Mawson, Perth Zoo Science Program Leader, Department of Biodiversity, Conservation and Attractions, Western Australian Government; and Samantha Setterfield, Associate Professor, School of Biological Sciences, University of Western Australia.

The support of the Harry Perkins Institute (a medical research facility in Perth, WA, and an educational outreach program for high school students) and Plant Energy Biology (PEB), who have so many innovative and collaborative projects happening around the globe) has been greatly appreciated.

Lastly, thank you to Jeanette Birtles and John Birtles (editors), Jane Fitzpatrick (proofreader), Helen Lydon (consultant) and Jane Brandenburg (reviewer). Their queries and advice have helped shape the book into a clearer and more up-to-date and reliable resource.

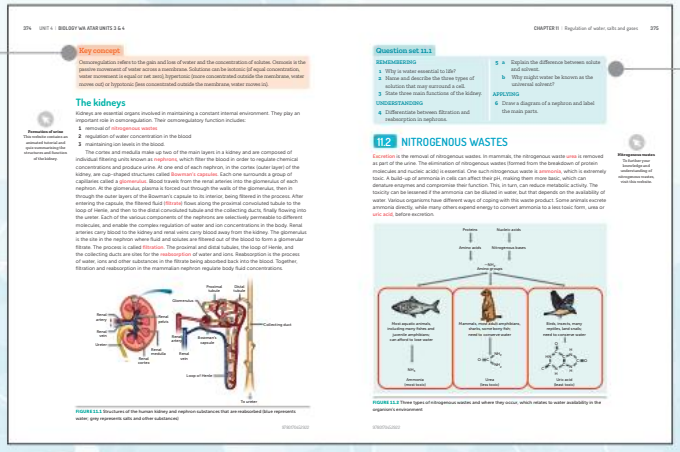
USING *BIOLOGY WA ATAR*

This new *Biology WA ATAR* (Units 1–4) series has been created and designed to fully meet the requirements of the Government of Western Australia, School Curriculum and Standards Authority (SCSA) Syllabus (2017). The series uses an enquiry-based approach to enhance and deepen learners' conceptual understanding and their ability to apply their knowledge. This educational package provides the resources necessary to address the Science Understanding concepts and Science as a Human Endeavour applications and Science Inquiry Skills described in the syllabus. The content is clearly illustrated, structured and presented, making it accessible to students of all levels so they can achieve maximum understanding and academic success.

Each page has been carefully planned so as to include all the information needed without appearing cluttered or overwhelming. Navigating through each chapter is easy. Activities and/or practical investigations have been included for each chapter, connecting the conceptual and the practical aspects of biology. Below is a list of the features to be found in each chapter so that students can navigate and fully utilise this resource.

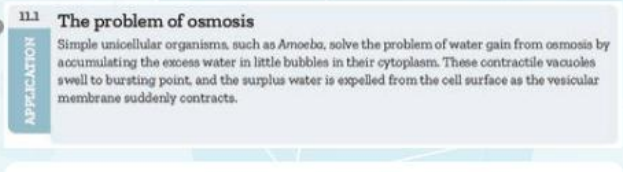
Each chapter begins with a **chapter opening page** including starter questions and the learning outcomes from the SCSA Biology ATAR Syllabus that will be covered in the chapter. This gives students the opportunity to monitor their own progress and learning.

Important ideas, concepts and theories are highlighted in **key concept boxes**, providing repetition and summary for improved assimilation of new ideas.



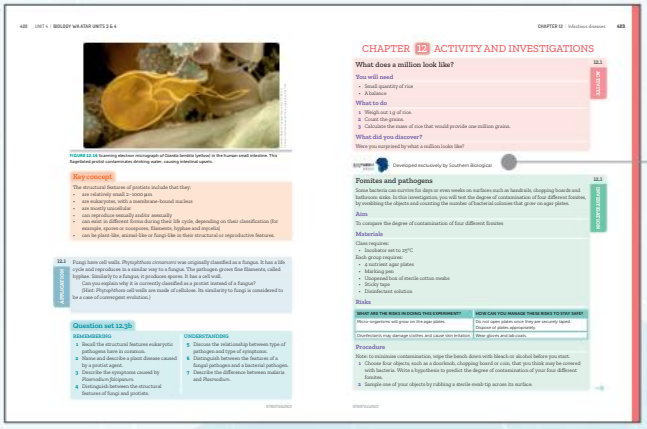
Question sets throughout each chapter enable formative assessment in bite-size chunks graded from Remembering to Creating, offering regular opportunities to recall new terms and review recent concepts.

Scientific literacy boxes discuss a scientific text or media item, encouraging students to use evidence to evaluate the claims and conclusions presented.

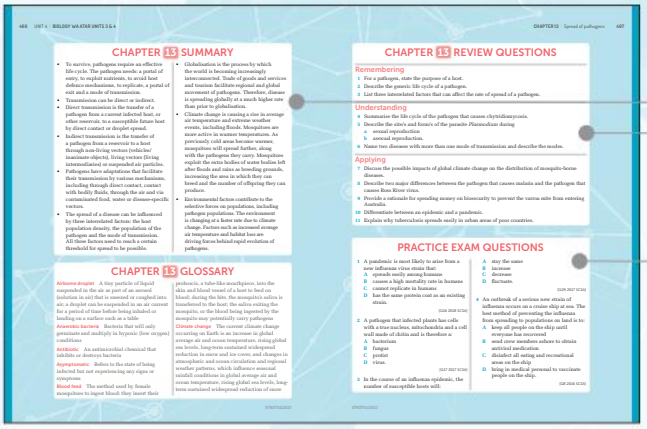


Application boxes offer opportunities for students to accurately *apply* models and scientific principles to complex systems in both familiar and unfamiliar contexts.

Case studies provide opportunities to see how science is applied using up-to-date, real-world and local Western Australian examples of context.



The **activities** and **investigations** offer opportunities to develop the Science Inquiry Skills listed in the SCSA Biology ATAR Syllabus. Southern Biological and Cengage have collaborated to ensure they are effective, exciting and current. Some of the investigations written by Southern Biological are exclusive to Cengage, and all investigations have been rigorously stress-tested by Southern Biological to ensure that they will work in the classroom. Some investigations have **taking it further** ideas at the end, suggesting how to extend or adapt them for further study.



Chapter summary sections and a **glossary** of important terms introduced in the chapter support revision and self-reflection. **Chapter review questions** provide, in larger chunks, further scaffolding for summative assessment, including Creating questions. **Practice exam questions** (WACE exam questions) give practice in answering exam questions specifically related to the content students have been studying.

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Worksheet

Weblink

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UNIT 3

CONTINUITY OF SPECIES

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1 SCIENCE INQUIRY SKILLS

CHAPTER 1 CONTENT

By the end of this chapter, you will be able to use the following science inquiry skills.

SCIENCE INQUIRY SKILLS

- identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes
- design investigations, including the procedure(s) to be followed, the materials required, and the type and amount of primary and/or secondary data to be collected; conduct risk assessments; and consider research ethics, including animal ethics
- conduct investigations, including the use of probabilities to predict inheritance patterns, real or virtual gel electrophoresis, and population simulations to predict population changes, safely, competently and methodically for the collection of valid and reliable data
- represent data in meaningful and useful ways, including the use of mean, median, range and probability; organise and analyse data to identify trends, patterns and relationships; discuss the ways in which measurement error, instrumental accuracy, the nature of the procedure and the sample size may influence uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions
- interpret a range of scientific and media texts, and evaluate models, processes, claims and conclusions by considering the quality of available evidence, and use reasoning to construct scientific arguments
- communicate to specific audiences and for specific purposes using appropriate language, nomenclature, genres and modes, including scientific reports

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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1.1 INVESTIGATIONS

Investigations use a scientific process to answer a question, explore an idea or solve a problem. They require activities such as planning a course of action, collecting data, interpreting data, reaching a conclusion and communicating about these activities. They can involve observation, data collection, research, field work, laboratory experimentation and manipulation of simulations. Investigations are central to our understanding of the biological world.

Sometimes an important advance in science begins with a lucky accident. For example, after hearing from milkmaids that people who contracted cowpox (a relatively harmless disease picked up after working with cattle) were protected from deadly smallpox, the British physician Edward Jenner effectively kickstarted the science of vaccination. Jenner used samples from open cowpox sores on a dairymaid's hands to **inoculate** a young boy and protect him against smallpox. In this process, he introduced a microorganism that caused a mild form of the disease, and it resulted in the boy developing immunity. However, nearly 100 years would pass and significant research would be required before scientists were able to show that microorganisms caused infectious disease. Lucky accidental discoveries like this may begin a new field of research, but they need to be followed up by carefully planned investigation.

Usually, advances in science come from a process of systematic observation and experimentation, inductive and deductive reasoning, and the formation and testing of hypotheses and **theories**. How these activities are carried out can vary greatly, but there are some common factors that we will explore below. The process by which science advances is known as the **scientific method**.

When an investigation has finished, it is good practice to check whether the findings align with current theories. Theory can be used to explain experimental results. A theory is 'a set of concepts, claims and/or laws that can be used to explain and predict a wide range of related observed or observable phenomena. Theories are typically founded on clearly identified assumptions, are testable, produce reproducible results and have explanatory power.'

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

Key concept

The main role of science is to observe, question and investigate the natural world. The scientific method enables science to proceed.

1.2 THE SCIENTIFIC METHOD

Investigations can vary greatly, so the generic steps for conducting an investigation will need to be adapted to the task.

The generic steps are:

1. Make an observation (or observations).
2. Ask questions about the observation(s).
3. Form a **hypothesis** and make predictions based on that hypothesis.
4. Test the hypothesis using a planned procedure that is reproducible, and uses the appropriate **models** and instruments.
5. Record results, analyse the data (usually by graphing it) and draw conclusions; accept or reject the hypothesis or modify the hypothesis if necessary.
6. Reproduce the experiment until there are no discrepancies between the observations and the modified hypothesis.
7. Discuss and evaluate the results and the procedure.

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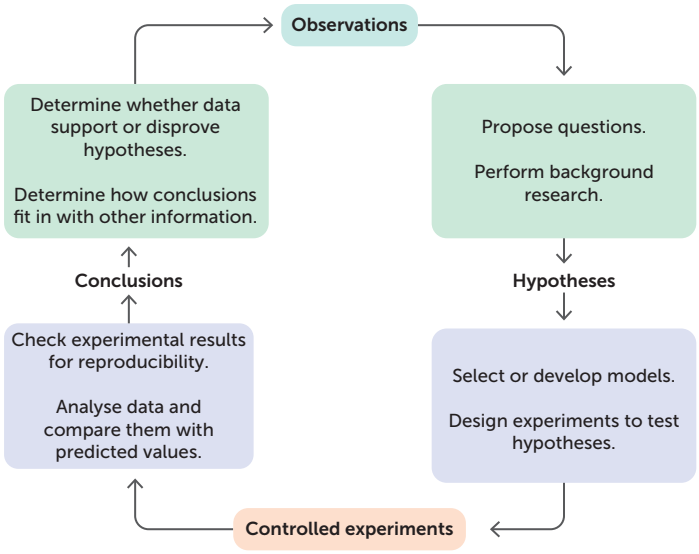

FIGURE 1.1 The scientific method

Researching and refining your question

After recording your observation(s) and formulating a question, the next step is to find out what is already known about the concepts. Use the Internet, your textbooks and the library to find out. Make sure you keep a record of the information that you find, as well as the sources of the information. You should start a **logbook** at this stage. Your logbook will contain the planning of your investigation, the raw data you obtain, and the draft of your report. Good record keeping is important in scientific research, and it begins at this stage of the investigation.

Read a range of scientific media and texts. Published journal articles (e.g. CSIRO articles) can be helpful. Be critical of what you read. Do not assume that everything you read online, or even in books, is true. Evaluate the claims and conclusions, and check that they were written relatively recently and have not been superseded by more recent research. Scientists spend a lot of time reading other scientist's published work so as to build on current knowledge. For example, scientists working on medications and vaccines for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing COVID-19, read widely about other pathogenic coronaviruses affecting humans, including SARS-CoV-1 and the Middle Eastern respiratory syndrome coronavirus (MERS-CoV). Immunologists at Harvard Medical School demonstrated in 2003 that SARS-CoV-1 used its spikes to penetrate cells. In 2020, scientists at the Chinese Academy of Sciences confirmed that SARS-CoV-2 attaches to the same receptor. Scientists working on the SARS-CoV-2 vaccine are using previous knowledge and recently developed vaccine technology (such as recombinant protein vaccines) to develop a safe and effective vaccine (Figure 1.2, page 4).

When you are conducting an investigation, you may find examples of similar investigations to the one you are thinking of. It is a good idea to look at these, so you can learn from the experience of other researchers. However, in general, it is better not to try to replicate or copy someone else's investigation exactly. If you do decide to replicate someone else's investigation, then you need to acknowledge and carefully reference their work. See the section on referencing (page 16). If you do not do so, it is **plagiarism**. This is a very serious form of academic misconduct.

Australian Academy of Science
This is a useful resource for up-to-date science news.

Australian Society for Biochemistry and Molecular Biology
This resource aims to promote education and research in biology.

CSIRO (Commonwealth Scientific and Industrial Research Organisation)
This website contains useful information on all fields of scientific research, including resources for students and teachers.

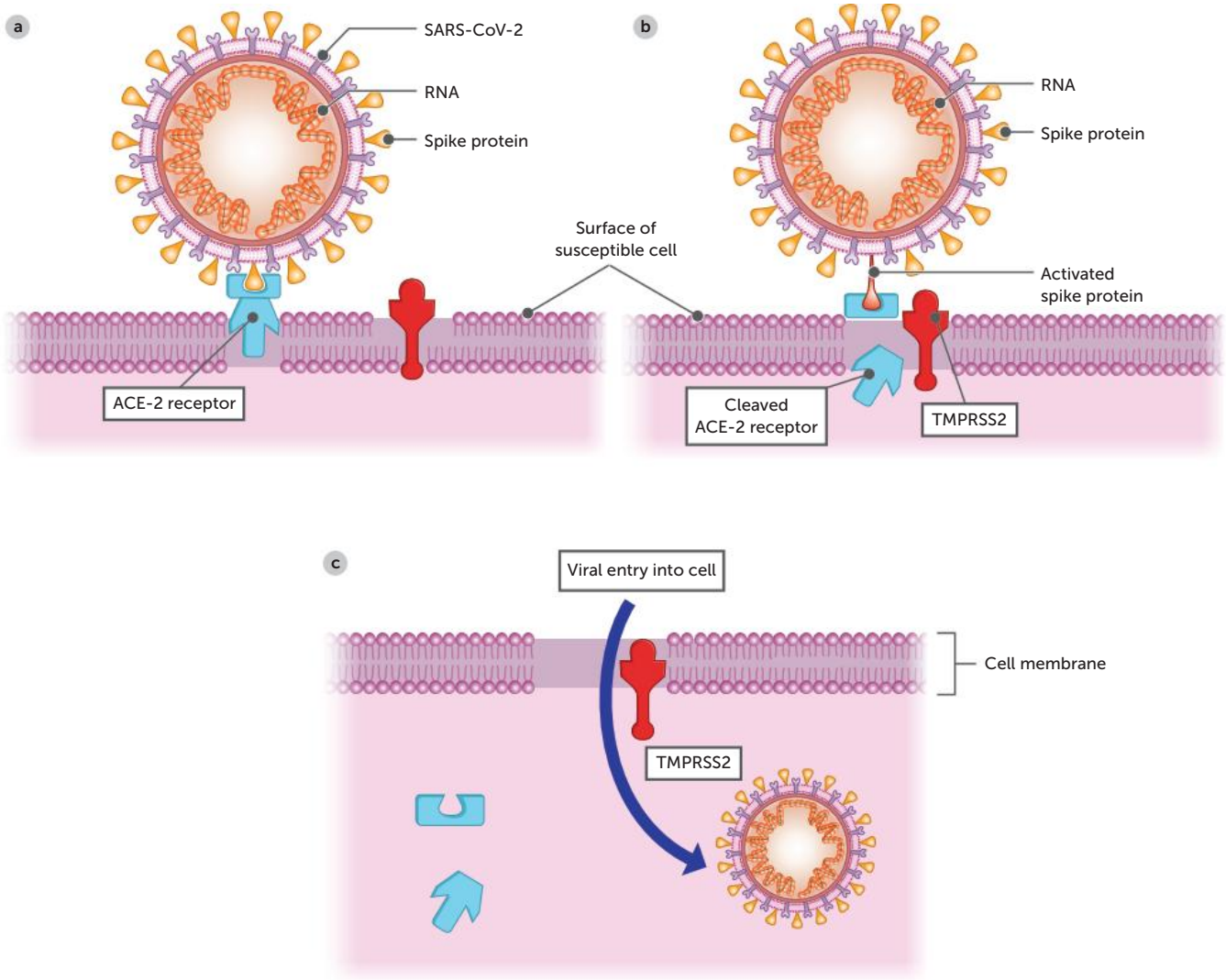

FIGURE 1.2 Scientists have found that the protein spikes on SARS-CoV-2 penetrate a cell by attaching to a host cell surface receptor protein called the ACE-2 receptor. The enzyme TMPRSS2 facilitates virus entry. They are using this knowledge to develop an effective vaccine.

Forming a hypothesis

A hypothesis is a scientific statement, based on the available information, that can be tested by experimentation. It can be thought of as 'an educated prediction'. It should describe an expected relationship between the independent and **dependent variables** in observable phenomena. Once you have decided on your **research question**, what you are trying to find out, you need to turn it into a hypothesis.

If your research question is in the form of 'What effect does a new fertiliser have on root growth?' it can be turned into a hypothesis such as, 'If a new fertiliser is applied to a plant, then the **rate** of root growth will increase'.

If the hypothesis is written correctly, the independent and dependent variables should be easy to identify. Usually, an investigation will have one dependent variable and one **independent variable** in each trial. A **variable** is a factor that can change. There may be several factors that can cause change in a particular variable. To conduct a trial that produces valid results, only one of these factors, the independent variable, should be manipulated (changed). The other factors, which need to be kept consistent between the **control group** and the experimental group, are called the

controlled variables. The factor that changes as a result of the change in the independent variable is called the dependent variable. It is the set of changes in the dependent variable that is observed, measured and recorded and which becomes the investigation's results. The results will either support or not support the hypothesis. A sound hypothesis is **falsifiable**, and results that do not support the hypothesis can be of as much value as those that do in accumulating scientific knowledge.

Even if your hypothesis meets these criteria, do not be surprised if you change or modify it during the course of your investigation. In scientific research, the question you set out to answer is often only a starting point for more questions.

For the hypothesis 'If a new fertiliser is applied to a plant, then the rate of root growth will increase', are you able to determine the independent and dependent variables? (The term rate refers to change in a quantity, usually per unit time. Root growth rate is likely to be measured in millimetres per day.) In the first half of the hypothesis, we see that the factor to be varied by the investigator is the type of fertiliser. This is the independent variable. In the second half of the hypothesis, we see that the factor to be observed and measured is the rate of root growth, the dependent variable. A controlled variable might be the amount of fertiliser added.

Key concept

A research question informs a hypothesis. A hypothesis describes the relationship between the independent and dependent variables.

Planning

Keep the hypothesis and the purpose of the investigation clearly in mind during the planning of the procedure. Your predictions about what you think may happen will help with your planning. It is helpful to ask yourself some questions first.

- What data will you need to collect?
- What materials and equipment will you need?
- When and where will you collect the data?
- If you are working in a group, who will collect the data?
- Who will be responsible for record keeping?
- How will the data be analysed?

The data that you collect will always include **primary data**, and will usually include **secondary data**. Secondary data are data collected by a person or group other than the person or group using the data. Primary data are data collected directly by the person conducting the investigation.

Consider how you will analyse the data. Will you need access to specific software, such as a graphing or statistics package? Keep a record of your planning. This should go in your logbook. Writing down what you plan to do, and why, will help you stay focused during the investigation. If you are working in a group, then a record of what each person agrees to do during the investigation can be very important.

When variables have a numerical value, you make **quantitative measurements**. You

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FIGURE 1.3 These seedlings are being grown under consistent conditions to control the independent variables.

measure each numerical value in the appropriate units. For example, you might measure root length in centimetres, or the weight of roots in grams.

Continuous variables can take any possible value, usually within a particular range. Length, time and current are continuous variables. In the root growth example, root length is a continuous variable because it can take any value within a range. A variable that can take only fixed values is called a **discrete variable**. Data for discrete quantitative variables (e.g. number of electrons in an atom) are usually whole numbers because the quantity cannot be broken into fractions. In the root growth example, the number of roots is a discrete variable.

In some investigations, you may have **qualitative measurements** as data. Qualitative data can include words or descriptions and can at times be subjective. For example, a chemical reaction may lead to a colour change. You would usually describe the colour in words, such as 'pink' or 'green', rather than using a number. Sometimes you use a combination of qualitative and quantitative data. For example, you may describe roots as reaching a maximum length in centimetres (quantitative), but growing in a particular direction or pattern (qualitative).

Once you have decided on the variables you will be measuring, you will be able to identify the equipment and other resources you will need.

Risk assessment

You may be required to complete a risk assessment before you begin your investigation. Even if this is not a requirement, it is a good idea to think about risks. You need to consider three things.

1. What are the possible risks to you, to other people, to property and to the environment?
2. How likely is it that there will be an injury or damage?
3. If there is an injury or damage, how serious are the consequences likely to be?

A 'risk matrix', such as Table 1.1, can be used to assess the severity of a risk associated with an investigation. The consequences are listed across the top, from negligible to catastrophic, and the likelihood of each consequence occurring increases as you look down the rows. A negligible consequence may be getting clothes dirty or a very minor injury such as a scratch. A marginal consequence might be a bruise from falling off a bike, or a broken branch in a tree. A severe consequence could be a more substantial injury or a broken window. A catastrophic consequence could be a death or the release of a toxin into the environment. In general, you need to ensure that your investigation is low risk. You can use a risk matrix either for individual identified risks, or for the investigation overall.

TABLE 1.1 Matrix for assessing severity of risk

CONSEQUENCES → LIKELIHOOD ↓	NEGLIGIBLE	MARGINAL	SEVERE	CATASTROPHIC
Rare	Low risk	Low risk	Moderate risk	High risk
Unlikely	Low risk	Low risk	High risk	Extreme risk
Possible	Low risk	Moderate risk	Extreme risk	Extreme risk
Likely	Moderate risk	High risk	Extreme risk	Extreme risk
Certain	Moderate risk	High risk	Extreme risk	Extreme risk

Once you have considered what the possible risks are, you need to think about what you will do about them: what you can do to minimise them, and how you would deal with the consequences if something did happen. This may be as simple as deciding to 'Always wear a lab coat, gloves and safety glasses'. You can use a risk assessment table similar to Table 1.2.

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TABLE 1.2 Example of risk assessment

WHAT ARE THE RISKS INVOLVED IN DOING THIS EXPERIMENT?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
The fertiliser might be spilled on clothes or skin during application.	Wear a lab coat, gloves and safety glasses. Clean up spills immediately.

Safe use and disposal of biological material

When dealing with many biological materials, it is important to be aware of safe handling and disposal. For example, when growing known or unknown microbes on agar plates, it is important to use safe sterile techniques (discussed below) and to wear a lab coat, gloves, safety glasses and, if required, face mask. Treat all microbes on agar plates as potentially pathogenic, and **autoclave** used plates before disposing of them. An autoclave is a machine that can heat an object to very high temperatures. It is sometimes used to kill pathogens.

Animal ethics

Activities that affect living organisms need to comply with the *Australian Code for the Care and Use of Animals for Scientific Purposes*.

The main thrust of ethics is treating animals, other people and the environment with care and respect. If your investigation will be using humans, then you need to make sure you do not harm them, either physically or psychologically. If you are working with animals, then there are animal ethics to consider. The welfare of animals used for the purposes of research is legislated by state and federal laws, and respect for all animals (vertebrate and invertebrate) used in research is of the utmost importance. When using animals for research, scientists must adhere to the '3Rs'. These are:

- **Reduction** alternatives: methods that obtain comparable levels of information from the use of fewer animals in scientific procedures, or more information from the same number of animals.
- **Refinement** alternatives: methods that alleviate or minimise potential pain and distress, and enhance animal wellbeing.
- **Replacement** alternatives: methods that permit the given purpose of an activity or project to be achieved without the use of animals or with the use of non-sentient animals or animals of a lower sentient value (those that lack a nervous system).

iStock.com/Maksym Panchuk

FIGURE 1.4 The use of animals for research purposes is governed by state and federal laws.

Australian Code for the Care and Use of Animals for Scientific Purposes
Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. Read about animal ethics here.

CASE STUDY

Animal ethics

Anyone working with animals in Australia is bound by guidelines and laws to safeguard animals. Research is conducted on animals other than humans for several reasons: some animals have lower sentience; also, the time between generations is shorter in some animals, which reduces the waiting time for results. The precise meaning of **sentience** is contested in science and in philosophy, but it can be defined as the ability to experience consciousness, pleasure, self-awareness and pain. Some invertebrates (such as jellyfish) do not have a central nervous system that is as sophisticated as that of humans, and they are considered to have a lower sentience than humans.

RSPCA Australia promotes ethical treatment of animals as observing what should, rather than what could, be done to animals. Scientists should have an understanding of the pain physiology of the animals used in their research. They also need to be able to recognise normal and abnormal behaviour, so they can assess an animal's welfare and make an ethical decision about whether or not to continue a trial.

A model is 'a representation that describes, simplifies, clarifies or provides an explanation of the workings, structure or relationships within an object, system or idea'.

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

A model can be used to test outcomes that might be expected in the real world: for example, an animal that has a similar immune response to humans can be used to test a vaccine; computer simulations can be fed real-world data to generate predicted outcomes. Animals such as mice, guinea pigs, rats, pigs and non-human primates are commonly used as models for research because, as mammals, they share a lot of genetic code with humans and have a likelihood of responding to tests in a similar way. A range of vaccines, infectious agents and chemicals have been tested on animals such as these to find out the possible effects on humans.

Since the discovery of the susceptibility of ferrets (*Mustela putorius furo*) to influenza virus in the 1930s, they remain one of the most useful animal models for studying influenza infection. They have a similar respiratory system to that of humans, their lung physiology is similar, they are susceptible to similar viruses, and (unlike guinea pigs and mice) ferrets sneeze, experience fever and produce nasal discharge.

More recently, COVID-19 researchers have been using ferrets for the testing of infection and vaccines. The research team of Dr Rob Grenfell, Director of Health and Biosecurity, CSIRO, aims to understand how the infection progresses and how it behaves so as to be able to create an effective vaccine. However, ferrets feel pain in the same way we do, so strict ethical guidelines for the use of ferrets and other animals in Australian research laboratories must be followed. The guidelines are to be found in the *Australian Code for the Care and Use of Animals for Scientific Purposes*.

Key concept

When using animals for research, scientists must adhere to the 3Rs: reduction, refinement and replacement alternatives.

Procedure

The procedure is a planned set of steps enabling you to approach your research systematically. It is important to describe clearly what you plan to do (noting any modifications you make) so you can review the procedure later and communicate it to others. It is written in the past tense. A fellow student should be able to follow your procedure and reproduce the results you have recorded. Let's examine an exemplar procedure for a given hypothesis. The first task is identifying the independent variable (the variable that you will vary), the dependent variable (the variable you will measure) and the controlled variables (those you will keep constant). (Note: fungicides are chemicals that are used to treat fungal diseases, such as rust in plants. The symptoms of rust include orange-brown patches on the leaves of affected plants.)

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HYPOTHESIS	If a particular brand of fungicide is applied to the plant, then symptoms of rust will disappear.
INDEPENDENT VARIABLE	Type of fungicide
DEPENDENT VARIABLE	Extent of symptoms (orange-brown patches)
CONTROLLED VARIABLES	Volume of water used, time of day for watering plants, plant species, ambient temperate, soil type, light conditions
PROCEDURE	**1** One hundred plants of the same species infected with the same species of rust (fungus) were obtained. All individual organisms had a similar leaf area of orange-brown patches. **2** Fifty plants were treated with the fungicide and 50 plants were left untreated. **3** Other than presence or absence of the fungicide, the conditions listed as 'controlled variables' were the same for both the experimental and control groups. **4** After a set time, each plant was examined to check the number and size of orange-brown patches. **5** The results were recorded in an appropriate table. **6** Repeat trials were conducted, and again the results were recorded. Averages of the number and size of orange-brown patches were calculated for the fungicide group and the non-fungicide group.

Once you have written a procedure, a checklist is useful to ensure your procedure has been written correctly. Check that:

- you have a relatively large sample size
- the independent variable will be present or varied in the experimental group, but absent or kept constant in the control group
- all other variables will be kept constant
- data will be collectable within the time frame you have available
- the method for measuring the dependent variable and the units of measurement are clear
- multiple trials will be conducted and an average will be calculated.

Key concept

Good design of an investigation is systematic and clear. It includes a procedure, materials, data collection, risk assessments and ethical considerations.

Results: recording the data

The raw data should always be recorded directly into a logbook, unless it is recorded using data loggers connected to a computer. In that case, a printout of the data should be attached to the logbook, and the file name and location recorded. Make sure that you measure and record everything you will need for your analysis. Use appropriate units (e.g. centimetres for lengths and grams for weights). Note that the **accuracy** of your measurements will often be restricted by the accuracy of the instruments you use to take them. For example, a ruler may only have markings down to 0.1 cm. Make a note of these restrictions, as they may affect the accuracy of your final results, especially if the changes measured are very small.

If you are going to be collecting multiple data points, it is a good idea to record them in a table. Label the columns in the table with the name and units of the variables. Do not put the units in the table cells. The first column usually contains the measurements for the independent variable. Table 1.3 (page 10) provides an example of a table of data. These data were recorded during an investigation into the use of a specific fertiliser and its impact on the yield of a species of wheat.

TABLE 1.3 Results of investigation into soil fertiliser and wheat yield

FERTILISER APPLICATION (kg/ha)	TRIAL 1 YIELD (BUSHELS/ha)	TRIAL 2 YIELD (BUSHELS/ha)	AVERAGE YIELD (BUSHELS/ha)
0	29	31	30
25	40	42	41
50	51	49	50
75	60	58	59
100	70	70	70

Results: analysing the data

Once you have collected your data, you can begin to analyse it. Performing statistical calculations, such as finding the mean, median, range and probability, allows you to represent the raw data in a more meaningful and useful way.

Calculating mean, median and range

The **mean** or average of a set of numerical data is found by:

1. adding all the data points together
2. dividing by the number of data points.

The **median** of a data set is the value that has half of the data points above it and half below it. The median of a data set can be found by:

1. arranging the data points in ascending order
2. selecting the middle data point; or, if there is an even number of data points, taking the mean of the two central values.

The range describes the total spread of the data. It can be calculated by:

1. ordering the data from lowest to the highest
2. subtracting the lowest value from the highest value.

The **inter-quartile range** of a data set is the middle 50% of values in a data set when they are ordered from lowest to highest. It is found by:

1. dividing the ordered data set into two halves
2. finding the median of the lower half of the set (Q1) and the median of the upper half of the set (Q3)
3. subtracting the median of the lower half from the median of the upper half (Q3 – Q1).

Quartiles and inter-quartile range This resource works through an example of calculating inter-quartile range.

General graphing rules

The most reliable way to present patterns in data, and help identify the relationships between variables, is to plot a graph. Graphs show points of data for at least two variables. Because of their visual nature, graphs can reveal relationships or trends that statistics and data tables cannot.

1. When presented with quantitative data, you need to decide whether they are discontinuous (discrete) or continuous (measurable) data and select the appropriate type of graph to use.
2. Line graphs are used when the independent variable is continuous (can take any numerical value within the range of the data) and the dependent variable is quantitative (can be measured or counted).
3. If the independent variable provides discontinuous quantitative data or qualitative data, a column (bar) graph is usually selected. The bars in a column graph do not touch.
4. Histograms look like column graphs for continuous quantitative data, but the bars *do* touch. Each bar on the *x*-axis represents a range of values for the independent variable. The *y* value is the number of individuals associated with each range of *x* values.
5. Pie charts are an alternative way of displaying data for a qualitative independent variable and are used to show what percentage or fraction of the whole each category represents. The data add up to 100% or '1', respectively.

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6 Column (vertical bar) and bar (horizontal bar) charts are useful for comparing two data sets in which at least one data set is qualitative. Examples would be average root length varying with different types of fertiliser, or the number of endangered species in the different states and territories of Australia. The bars do not touch (see Figure 1.6, page 12). However, do not use a column or bar chart to try to show a mathematical relationship between variables.

7 The independent variable measurements are on the *x* axis. The axis is labelled, and the units are included in brackets [e.g. Time (years)].

8 The dependent variable measurements are on the *y* axis. Again, the axis is labelled and the units are included in brackets [e.g. Length (cm)].

9 Include a title to help the reader determine what the graph is showing. The title should include the variables involved and, when appropriate, the specific time over which the experiment was conducted (e.g. 'The effect of temperature on the amount of sugar dissolved in tea after 2 minutes').

10 The graph should take up more than 50% of the graph space provided. The scale should be worked out to maximise the filling of the graph space, while allowing some space for extrapolation of data. The data points should *not* extend outside of the given graph space. The intervals can be worked out by dividing the range of data by the number of available intervals on the axis. In Figure 1.7 (page 13), you will see an example of a set of data points for the investigation on soil fertiliser and wheat yield.

    The range of the data is 0 to 100 kg/ha on the *x*-axis and 0 to 70 bushels/ha on the *y*-axis. There are 12 graph intervals on the *x*-axis and 16 graph intervals on the *y*-axis. Allowing some room for extrapolation within the graph space available, using 10 of the 12 *x*-axis intervals for 100 units of data, and 14 of the 16 *y*-axis intervals for 70 units of data works well.

    If the number of intervals on the *y*-axis is 6, to allow some room for extrapolation, this could be reduced to 5 intervals. Each interval thus represents 20 kg/ha.

    As a guideline, use sensible interval values (such as 1, 2, 5, 10 or 50) to make the graph easy to plot and interpret. Poorly spaced scales can lead to inaccurate readings. Always start from zero. On rare occasions, to manipulate the scale to fit, you may need to 'break' the axis after zero and then select equal intervals for the scale after the break.

11 When showing multiple sets of data on the same set of axes, a key should be used. You can use symbols such as Δ, × or o. The name of the data set that each line on the graph represents can be written near it.

Graphs drawn in biological science

1 To see whether there is a trend, carefully rule short straight lines between each of the plotted data points.

2 Do not extend the line further beyond the minimum or maximum recorded data. However, if asked to extrapolate, you can rule a dashed line for a short distance beyond the last data point if the graph is approximately linear.

3 The title should include the independent and dependent variables and, if appropriate, the time period over which the experiment was conducted. It does not need to be brief. Words such as 'versus' or 'against' are just restating the axis labels and should not be used in graph titles. Instead, rephrase it for easier reading, as shown in Figures 1.5–1.8 (pages 12–13).

4 Do not draw a line of best fit unless you have good reason to expect there is a linear relationship between the independent and the dependent variables. The relationship may not be linear, the variables may not be controlled enough to produce a straight line, and there are often insufficient data points to be confident about the values in between.

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5 **Interpolation** involves reading points, other than data points, within the region in which you have data. It is a prediction made between two known data points. Linear **extrapolation** involves reading points other than data points in the region beyond your measured points. Interpolation is more reliable than extrapolation, because it involves a prediction within the range of the known data points. Linear extrapolation is an estimation determined by extending the last line, using the gradient of the last two data points if there is a relationship between the variables. For the purposes of this course, when a relationship between the independent and dependent variables has been established, extrapolation is done by extending the line joining the last two data points for a short distance with a dotted line. Note that many scientists, however, extrapolate by extending the line of best fit. If one of the last two points is an outlier, the gradient of the line connecting them will not be a good indicator of the trend. An **outlier** is a data point that does not fit the pattern shown by other measured data points. The line of best fit is a line ruled through the data points with an equal number of data points either side of it. It can be created mathematically for more accuracy, and published scientific papers often contain examples of this technique.

Data points and line graphs

Biology for life
This website contains some helpful advice on deciding the number of data points.

Graphing with Excel
After learning the skill of hand-drawing graphs, students interested in creating graphs using Excel can use these tools.

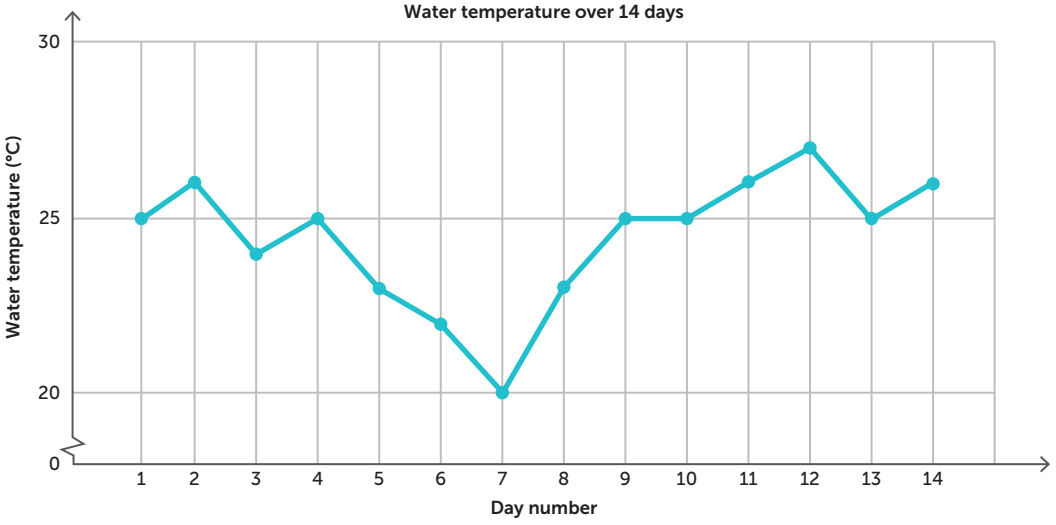

FIGURE 1.5 A typical line graph in a biological sciences ATAR course

Standard deviation
Standard deviation calculations can inform a scientist about the spread of the results. A high standard deviation indicates that the data points are quite varied from the average, whereas a low standard deviation indicates that most points are very close to the average.

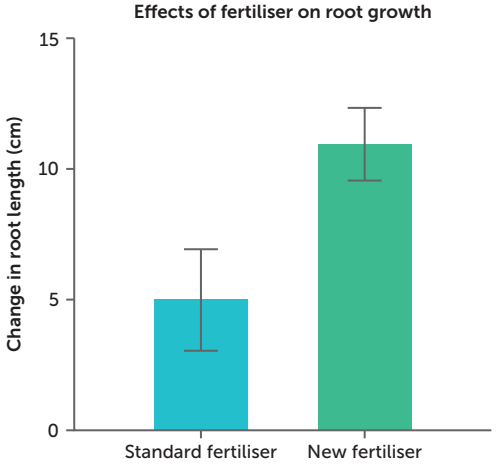

FIGURE 1.6 Bar graph of two data sets, with amount of uncertainty indicated by the error bar at the top of each data set

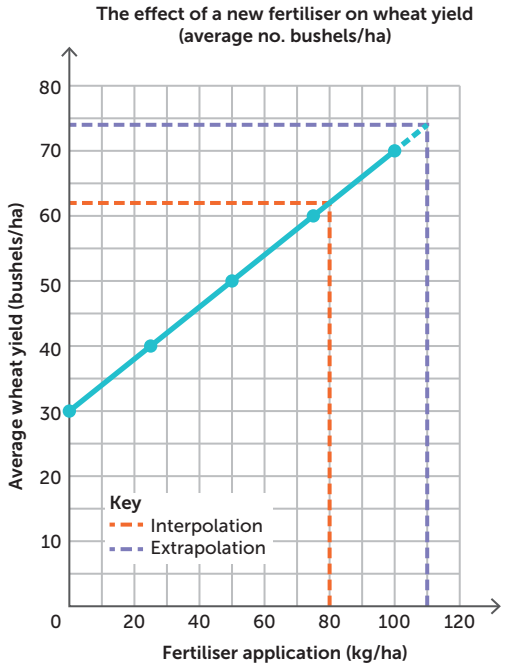

FIGURE 1.7 Graph demonstrating interpolation and extrapolation from the results of the investigation on soil fertiliser and wheat yield. Interpolation indicated 62 bushels/ha. Extrapolation indicated 74 bushels/ha.

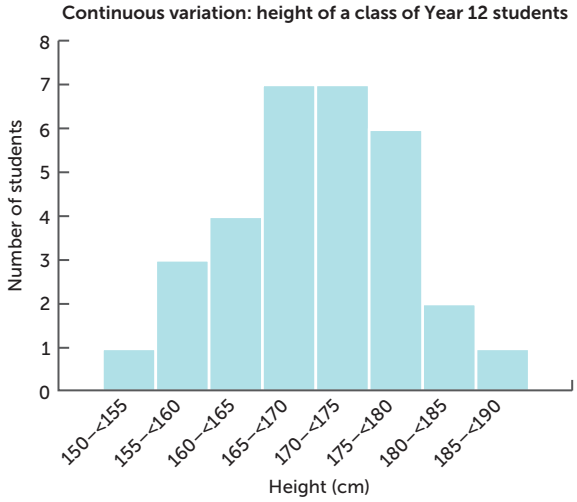

FIGURE 1.8 Histogram showing continuous variation in height

Key concept

Interpolation is prediction of a data point within the range of the known data points. Extrapolation is prediction of a data point outside the range of the measured data.

Discussion

The discussion needs to include an evaluation of the procedure and the results. It can be broken into sections such as the following.

1 *An evaluation of the reliability and validity of the procedure and the accuracy of the results*

You can almost always make suggestions to improve the procedure.

Reliability is the degree to which an assessment instrument or protocol consistently and repeatedly measures an attribute and achieves similar results for the same population. When you repeat an experiment and get the same results, the data are considered reliable.

Reliable data can be obtained by repetition and replication if the procedure is valid and good experimental technique is used. Repetition involves multiple trials within the same investigation or using a large sample size. Replication is the same investigation being conducted several times, possibly by more than one investigator. An average (mean) can often be calculated from the quantitative results obtained by both repetition and/or replication. This can reduce anomalous data/outliers.

Validity is the extent to which tests measure what was intended, and the extent to which data, inferences and actions produced from tests and other processes are accurate. Valid data can be achieved by identifying the variables that should be controlled and then controlling them. It can also be gained by the use of a control group. A control refers to a standard or group that is the same as the experimental group but for which the independent variable does not vary. The purpose of the control group is to ascertain that the cause of any change in the experimental group is due to change in the one independent variable.

Accuracy is the extent to which a measurement result represents the quantity it purports to measure; an accurate measurement result includes an estimate of the true value and an estimate of the uncertainty. It is the degree of closeness of a measurement of a quantity to its actual true value.

2 *A discussion of the ways in which measurement error may have affected the data*

Measurement error is the difference between the experimental result and its true value.

Systematic error, mistakes and random error can all contribute to measurement error.

Systematic error is error that is due to instrument accuracy and use; for example, the failure to zero an instrument before use or using the wrong type of instrument for the investigation. Reading an instrument from a *consistent* but non-90° angle can produce this type of error. A potometer may leak water and indicate a higher rate of transpiration than is correct. If a student *consistently* reads the volume of a liquid at the same but wrong part of the meniscus, there will be systematic error. Systematic error can shift measurements in a consistent direction. This will have an impact on the reliability of the results, because if the investigation is repeated without the error, the results will not be the same.

Mistakes, or *avoidable* measurement error, may arise from carelessness or incorrect use of an instrument. If a student knows to measure a water level at the bottom of the meniscus, but does not take care that their eye is level with the meniscus, avoidable measurement error can occur. Such error will also affect the results.

Finally, random error may occur. The only way to reduce this type of error is to increase the sample size or the number of trials.

3 *Future applications and implications of the investigation.*

Interpreting your results: conclusions

If your results support your hypothesis, then this can be stated in a conclusion, along with a statement about the relationship between the variables shown by the results.

A sample conclusion for the investigation on soil fertiliser and wheat yield could be as follows.

The results supported the hypothesis. As the fertiliser application increased from 0 to 100 kg/ha, the average number of bushels per hectare rose from 30 to 70. An increase in fertiliser application caused an increase in wheat yield.

There needs to be clear evidence for any relationship claimed in the conclusion. The evidence in this case will be of a quantitative nature, because the data recorded were quantitative. If you gather qualitative results, then key qualitative data are needed as evidence for the conclusion about the hypothesis.

If the results do not support the hypothesis, this can be stated in the conclusion instead. Possible reasons for this may be suggested in the discussion.

It may be that the nature of the procedure did not take into account all of the variables. For example, in the root growth experiments, it may be that the new fertiliser works best at a particular temperature, or over a longer time, or in conjunction with certain soil conditions.

It may be that the sample size used in the experiment was too limited to fully test the hypothesis. Thus, you might conclude that further experiments are required to increase the total sample size being tested.

Before you decide that the hypothesis was wrong, it is a good idea to check carefully that you have not made any mistakes or ignored any variables needing to be controlled.

Key concept

The discussion and conclusion critically evaluate the procedure and results of an investigation, discussing their reliability, validity and accuracy, and, when relevant, suggest ways the investigation could be improved.

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1.3 COMMUNICATING YOUR RESULTS

If research is not reported, then no-one else can learn from it. An investigation is not complete until the results have been communicated. The majority of scientific investigations are first communicated to others through the publication of written reports.

Writing reports

A report is a formal and carefully structured account of your research. It is based on the data and analysis in your logbook. However, the report is a *summary*. It contains only a small fraction of what appears in the logbook. Your logbook contains all your ideas, rough working and raw data. The report typically contains none of this.

A report consists of several distinct sections, each with a particular purpose. It usually includes the following:

- Introduction
- Procedure
- Results and analysis
- Discussion
- Conclusion
- Acknowledgements
- References
- Appendices.

Reports are always written in the past tense, because they describe what you have done.

Introduction

The introduction tells the reader why you did the investigation and what your research question and hypothesis is. This is the place to explain why your research is interesting or important.

The introduction also provides any background information needed to be able to understand the rest of the report. This is the place to summarise any existing theories. You need to do this to put your hypothesis into context. You should also summarise any similar investigations. All of this should be correctly referenced, as described in the section on referencing (page 16).

Procedure

The procedure describes what you did. It summarises what you measured and how you measured it, step by step. Write your procedure using sentences, not dot points. There should be enough detail for another student to be able to replicate the experiment. Remember that it needs to be written in past tense. For example, you would write, 'the length was measured', not 'measure the length'. Include any diagrams, such as apparatus set-ups, that are needed to make your procedure clear. The diagrams in your logbook will usually be rough sketches. The diagrams in your report should be very neat and carefully labelled. Flow charts can be useful for describing any procedures in which a series of steps has been followed. Each diagram should have a figure number, and you should refer to it within the text of your report. Position the diagram close to the relevant part of the text. Now is a good time to learn how to position figures neatly using your word processor software. When including images taken on a microscope, a scale bar and magnification must always be noted.

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Results and analysis

The results section is a summary of your results. It is usually combined with the analysis section, although they may be kept separate.

When you draw a table of results, the independent variable is usually positioned in the first column and the dependent variable in the second column. If a table has more than a few rows of data, it is better to represent that data in some other way. Usually this will be a graph. Think about what sort of graph would be appropriate. (Refer to pages 10–13 for guidance.) Do not use a column or bar chart to try to show a mathematical relationship between variables.

Discussion

The discussion should explain what your results mean. If you began with a research question, give the answer to the question here. If you began with a hypothesis, state whether or not your results support your hypothesis. If not, analyse your procedure and results to explain why. Was your hypothesis incorrect? Or was the procedure or model used not suitable for the investigation?

If there are any implications of your work, such as implications for better agricultural processes or the design of better medicines, put them here.

Conclusion

Refer to Interpreting your results: conclusions (page 14).

References

When writing a report that includes information from existing literature, use a citation to indicate within the report that you are using someone else's information or ideas, then include a list of **references** at the end. A reference list acknowledges the sources you used, which helps you to avoid plagiarism and strengthens your arguments. References can include journal articles, books, book chapters, websites, newspaper articles, conference proceedings, podcasts etc.

APA referencing by Murdoch University
A science bibliography usually uses APA referencing.

American Psychology Association (APA) referencing is commonly used in science reports. The APA citation style within the body of the report is an 'author–date' style: write, for example, '(Tian and Castillo, 2016)', 'Roberts et al., 2019' or 'Tian and Castillo (2016) observed …'. 'et al.' means there are more than two authors.

The reference list allows readers to locate sources easily and usually follows the chosen referencing style, APA in this case. Write the surname of the author(s) followed by their initials with a comma between authors, then the year of publication within parentheses, the title, the publisher and the place of publication. For example:

Tian M, Castillo TL (2016) *Solar heating uptake in Australia: rates, causes and effects*. Energy Efficiency Reports. Report no. 10, The Department of Sustainability and Environment, Canberra.

When a website without an author is used, the listing looks like this:

ABC News. (2003) *$250 m funding boost for malaria vaccine*. Retrieved from https://www.abc.net.au/news/2003-09-22/250m-funding-boost-for-malaria-vaccine/1482220

Other ways of communicating your results

You may want to present the results of your investigation in another format. Scientists communicate their findings in many ways: posters, seminars, journal articles, reports and websites. Scientists usually use more than one means to communicate their research when it is of particular interest.

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Look at examples of articles in the scientific literature and the popular media. This will give you an idea of how different styles are used in different contexts. Think about the purpose of the communication. Is it to inform, to persuade or both? What sort of language is used?

Think about your audience and use appropriate language and style. A poster is not usually as formal as a report. A website may be more or less formal, depending on the audience.

Multimedia presentations
- PowerPoint®
- Canva®

Oral presentations
- Informative
- Persuasive

Journal articles
- Research
- Case study

FIGURE 1.9 Examples of formats for reporting an investigation

FIGURE 1.10 A poster session is a common way to present scientific findings at a conference.

WS
Chapter 1 Activity sheet

CHAPTER 1 SUMMARY

- Science involves investigations. Prior to an investigation, scientists: identify, research and construct questions; propose hypotheses; and predict possible outcomes.
- The scientific method is used to conduct an investigation. The sequence followed in an investigation may include: observation, question, research, hypothesis, prediction, procedure, results, graph, discussion and conclusion.
- Scientists read and interpret a range of scientific and media texts. Scientists use critical thinking to evaluate claims and conclusions, which leads them to construct logical scientific arguments.
- Primary and/or secondary data are collected to test a hypothesis.
- Prior to an investigation, a scientist should conduct risk assessments and consider research ethics.
- Any investigations that use animals must consider the three Rs of animal ethics: reduction, refinement and replacement.
- Scientists write a clear procedure to ensure the investigation collects accurate, valid and reliable data.
- Raw data are recorded in a logbook and tables, and analysed using statistical methods (e.g. mean, median, range, probability and standard deviation).
- Data can be displayed in a meaningful way by plotting graphs.
- The discussion and conclusion sections of a written report evaluate ways in which measurement error, instrumental accuracy, the nature of the procedure or the sample size may influence uncertainty in and limitations of the data.
- Written reports are often used by scientists to communicate their investigations. Posters, oral and multimedia presentations at conferences, and journal articles are also used.

CHAPTER 1 GLOSSARY

Accuracy The extent to which a measurement result represents the quantity being measured; an accurate measurement result includes an estimate of the true value and an estimate of the uncertainty

Autoclave A device used to sterilise equipment, reagents or contaminated waste; autoclaves work by subjecting contents to pressurised steam at 121°C for a set time

Continuous variable A variable that is able to take any value within a range; length, time and temperature are examples of continuous variables

Control group A comparison group that is as similar as possible to the experimental group, except for the variable being tested (the 'independent variable'); the independent variable is absent or unchanged in the control group

Controlled variable A variable that is controlled by the experimenter and kept constant during the experiment

Dependent variable A variable that changes as a result of changes to the independent variable

Discrete variable A variable that may only take certain values, e.g. number of individuals, or number of legs on an animal

Extrapolation Extension beyond the measured range of data to predict or construct new data that has not been measured

Falsifiable Able to be disproved

Hypothesis (plural hypotheses) A scientific statement based on the available information that can be tested by experimentation ('an educated prediction'). It may describe an expected relationship between the independent and dependent variables based on observed phenomena

Independent variable A variable intentionally varied by an experimenter to see what the outcome will be for another variable (the 'dependent variable')

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Inoculate To inject a harmless form of a disease into an organism to induce immunity without causing the disease

Interpolation Predicting or constructing a new data point that has not been measured but is within the range of the measured data

Inter-quartile range The middle 50% of values in a data set when they are ordered from lowest to highest, found by subtracting the median of the lower half of the values from the median of the upper half of the values

Investigation A scientific process of answering a question, exploring an idea or solving a problem; it requires activities such as planning a course of action, collecting data, interpreting data, reaching a conclusion and communicating these activities. Investigations can include observation, research, field work, laboratory experimentation and manipulation of simulations

Logbook The record of an experiment or investigation kept by the scientist performing the experiment; it is a legal record of the experiments and their results

Mean The average of a set of values, found by adding all the values together and dividing by the number of values

Measurement error The difference between the measurement result and a currently accepted or standard value of a quantity

Median The central value in a data set, found by placing the values in ascending order and selecting the middle value or, if there is an even number of values, taking the average of the two central values

Model A representation that describes, simplifies, clarifies or provides an explanation of the workings, structure or relationships within an object, system or idea

Outlier A data point that does not fit the pattern shown by other measured data points

Plagiarism Presenting someone else's work, including their words or ideas, as your own

Primary data Data that you have measured or collected yourself

Qualitative measurement A measurement with descriptive or non-numerical results

Quantitative measurement A measurement with numerical values

Rate Commonly, total change in a variable divided by the time taken; it is a mathematical ratio of two measurements with different units; sometimes the second variable is not time – a rate can be the change in a variable per unit of length or mass, etc.

Reduction Using only the minimum number of animals needed to satisfy the statistical requirements of research

Reference The source of a specific piece of information or quotation

Refinement Decrease in the incidence or impact of procedures applied to animals that are needed for research

Reliability The degree to which an assessment instrument or protocol is able to consistently and repeatedly measure an attribute and achieve similar results for the same population

Reliable data Data that have been judged to have a high level of reliability

Replacement Substitution of insentient materials for conscious living animals

Research question The specific question that a particular experiment or investigation is attempting to answer

Scientific method A process of systematic observation and experimentation, inductive and deductive reasoning, and the formation and testing of hypotheses and theories

Secondary data Data or information that have been collected by someone else

Sentience The capacity to feel and experience emotions such as pain, fear, joy and pleasure

Theory A collection of models and concepts that explains specific systems or phenomena; scientific theories allow predictions to be made and hence are falsifiable

Validity The extent to which tests measure what was intended, and the extent to which data, inferences and actions produced from tests and other processes are accurate

Variable Something that can change or be changed, as distinct from a constant, which does not change

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CHAPTER 1 REVIEW QUESTIONS

Remembering

1 A scientist in Australia observed the effects of a potential vaccine on some ferrets and recorded the data in a table. The Australian scientist sent the data to a scientist working for the World Health Organization. Which scientist collected primary data and which scientist collected secondary data? State the criteria for your selection.

2 Scientists have studied the effects of the amount of annual/seasonal rainfall (mm) on agricultural beef production (measured in a standard cattle unit). Rainfall varies in different areas of WA. Some pastoralists are exploring the use of supplementary irrigation systems to grow improved tropical pastures such as sorghum and cowpea for the production of hay to feed cattle during periods of low rainfall.

For the research already conducted on beef production, state the independent and dependent variables.

Understanding

3 If one student reads 27.5°C and another student reads 27.8°C when taking the temperature of the same solution, suggest two strategies they could adopt to decrease the error, and thus increase the reliability of their readings.

Applying

4 Identify whether each of the following statements is a prediction, hypothesis, inference or conclusion.

- **a** I deduce that a Year 12 student with dark circles around his eyes has had little sleep.
- **b** If the amount of regular homework completed decreases, then a student's assessment marks will decrease. (The trend is going to be investigated with an experiment.)
- **c** A Year 12 student will know more about biology than a Year 2 student.
- **d** It was found that 93% of students who handed in homework regularly achieved a satisfactory or above grade. This supported the hypothesis that regular homework has a positive impact on assessment scores.

5 In recent years, WA has exported more than 350 000 live cattle per annum. WA has an excellent clean animal health status and a National Livestock Identification System that allows the strictest biosecurity protocols to be practised. For the time period of 2016/17, the main export markets for WA live cattle were Indonesia, Vietnam, Israel, Turkey and Malaysia.

Construct a pie chart for the following data. Use a ruler and a protractor if you are drawing by hand, or use a software program such as Excel.

Indonesia 46%

Vietnam 18%

Israel 18%

Turkey 15%

Malaysia 2%

Other markets 1%

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PRACTICE EXAM QUESTIONS

Questions 1 and 2 relate to the information below.

Some businesses provide life insurance for pets. Data were obtained from one business on the cause of death of insured cats over the period 1999–2006. The data are shown in the table below.

CAUSE OF DEATH	NUMBER OF INSURED CATS
Kidney failure	907
Traffic accidents	411
Other accidents	153
Skin cancers	165
Blood cancers	235
Other cancers	128
Viral infections	407
Bacterial infections	24
Heart disease	421
Hormonal disease	98

1 What proportion of cats in the data above died from cancers?

A 0.04
B 0.12
C 0.14
D 0.18

[Q19 2019 SCSA]

2 Which of the following is a valid conclusion from the data?

A Few owners vaccinate their cats against viral infections.
B Kidney failure is the most common cause of death in uninsured cats.
C Infectious diseases killed more insured cats than heart disease.
D Cat owners mainly insure their cats when the cats are ill.

[Q20 2019 SCSA]

3 Biologists counted the number of seeds produced by the flowers on five plants infected with a disease. The data are shown in the table. Note that some plants had more flowers than others.

The numbers of seeds per flower for each of the five plants were as follows.

	FLOWER 1	FLOWER 2	FLOWER 3	FLOWER 4
Plant 1	17	22	18	18
Plant 2	12	2	9	–
Plant 3	40	16	13	14
Plant 4	21	18	–	–
Plant 5	41	–	–	–

The mean number of seeds per flower for Plant 2 is:

A 5.8
B 7.7
C 14.5
D 18.8.

[Q16 2017 SCSA]

4 In an experiment, the factor that is manipulated by the experimenter is called:

A a control
B a dependent variable
C an independent variable
D a replicate.

[Q22 2017 SCSA]

5 A man's resting heart rate was measured at weekly intervals over a 5-week period during which the man undertook fitness training. The data are tabulated below.

WEEK	RESTING HEART RATE (BEATS PER MINUTE)
1	84
2	80
3	71
4	69
5	66

These data indicate that the man's resting heart rate:

A was above 90 beats per minute before the experiment began
B will drop below 60 beats per minute if the fitness training continues
C declined at the fastest rate between weeks 2 and 3
D increased by 20 beats per minute over the 5 weeks.

[Q6 2016 SCSA]

6 An increase in the sample size of an experiment will:

- **A** increase the reliability and validity of the experiment
- **B** increase the reliability of the experiment but not the validity
- **C** increase the validity of the experiment but not the reliability
- **D** not affect the reliability or validity of the experiment.

[Q12 2016 SCSA]

7 The water flea *Daphnia* is a small crustacean that lives in fresh water. When *Daphnia* are examined under low magnification with a microscope, the heart is clearly visible and the beats can be counted. A biologist wanted to study the influence of temperature on the heart rate of *Daphnia*. He collected 50 *Daphnia*, randomly assigned 10 individuals to each of five temperatures and measured the heart rate of each individual after 15 minutes at the assigned temperature. The results are shown in the table below.

	HEART RATE OF 10 DAPHNIA (BEATS PER 20 s)	
TEMPERATURE (°C)	MEAN	RANGE
2	59	39–85
10	119	82–151
20	142	92–234
30	257	178–328
40	401	206–596

a Graph the mean heart rate of the *Daphnia* against temperature. (6 marks)

b **i** Estimate the heart rate for *Daphnia* at 15°C. (1 mark)

ii Estimate the heart rate for *Daphnia* at 45°C. (1 mark)

iii In which estimate do you have the greater confidence? Give a reason for your answer. (2 marks)

c **i** What is the independent variable in this study? Give a reason for your answer. (2 marks)

ii State one way of improving the reliability of the study. (1 mark)

iii Propose a hypothesis for the study. (1 mark)

d Explain why the biologist waited for 15 minutes before measuring the heart rate of the *Daphnia* at the assigned temperature. (3 marks)

e One of the *Daphnia* had a heart rate of 208 beats per 20 seconds. A biologist concluded that this *Daphnia* must have been assigned to a temperature of 30°C. Evaluate this conclusion. (4 marks)

[Q32 2019 SCSA]

8 Soil salinity is a problem in agricultural areas because many crop species cannot tolerate high concentrations of salt. Biologists conducted an experiment to investigate why barley is more tolerant of soil salt than lupins are. They germinated 90 barley plants and 90 lupin plants and grew the plants in identical conditions except for variation in the concentration of salt in the soil. After 6 weeks, the biologists measured the concentration of salt in the xylem tissue of the plants. The results are shown in the table below.

	MEAN SALT CONCENTRATION IN THE XYLEM (mmol L^{-1})	
SALT CONCENTRATION IN SOIL (mmol L^{-1})	BARLEY	LUPINS
0	0	0
25	2	No data
50	2	3
75	No data	7
100	5	6
125	No data	6
150	4	No data
175	No data	59
200	7	No data

a Graph the mean salt concentration found in the xylem for both barley and lupins against the salt concentration in the soil. (6 marks)

b **i** Estimate the mean xylem salt concentration for barley for a soil salinity of 175 mmol L^{-1}. (1 mark)

ii Estimate the mean xylem salt concentration for lupins for a soil salinity of 150 mmol L^{-1}. (1 mark)

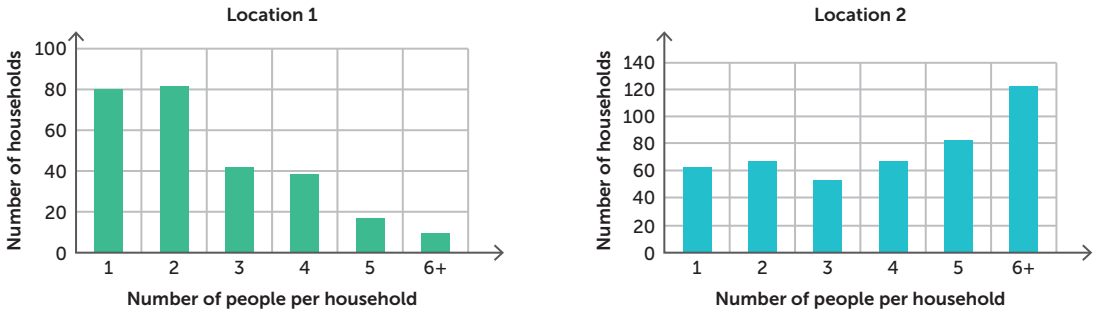
iii In which of the above estimates do you have more confidence? Give a reason for your answer. (2 marks)

c Explain why the biologists grew the plants under identical conditions except for the variation in soil salinity. (3 marks)

d Explain why the biologists used 90 plants of each species rather than 18. (3 marks)

[Q34 2018 SCSA]

9 A group of biologists developed a model for predicting the spread of influenza in human populations. As a part of this, they collected data on the number of individuals per household in two locations, which are shown in the figure below.



Compare the number of people per household in the two locations. Use the data provided in the graphs to support your answer. (4 marks)

[Q32a 2016 SCSA]

10 Biologists suspected that a species of fruit fly was developing resistance to a commonly used insecticide. They collected 1000 fruit flies from an orchard sprayed regularly with this insecticide. In the laboratory, they sprayed the fruit flies from the orchard with the recommended dose of insecticide and measured the percentage survival of the flies over the next 100 hours. At the same time, they also sprayed a group of 1000 laboratory-reared fruit flies of the same species that had never been exposed to insecticide and recorded their percentage survival over the next 100 hours. Fruit flies in both groups were kept under identical culture conditions. The data are shown below.

TIME SINCE SPRAYING (HOURS)	% FRUIT FLIES FROM THE ORCHARD SURVIVING	% LABORATORY-REARED FRUIT FLIES SURVIVING
0	100	100
20	97	8
40	51	4
60	50	2
80	49	2
100	49	0

a Graph the percentage of fruit flies surviving over time for both the fruit flies from the orchard and those from the laboratory. (6 marks)

b i State a hypothesis for the fruit fly experiment. (2 marks)

ii Does the fruit fly experiment have a control? Explain your answer. (3 marks)

c **i** Calculate the number of flies from the orchard that died between 20 and 40 hours after being sprayed. Show your workings. (2 marks)

ii Using your graph, estimate the time by which 50% of the fruit flies from the laboratory had died. (1 mark)

iii Explain how you could modify the experiment to improve the accuracy of the estimate of the time by which 50% of the fruit flies from the laboratory had died. (2 marks)

[Q33 2017 SCSA]

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2 PROCESSES FOR THE CONTINUITY OF LIFE

CHAPTER 2 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Can you describe the processes required for the genetic code to be transferred to the next generation?
2 How do single cells and whole organisms transfer their genetic material to daughter cells? Is the transfer similar?
3 Do all cells duplicate their chromosomes before division?

SCIENCE UNDERSTANDING

» continuity of life requires the replication of genetic material and its transfer to the next generation through processes, including binary fission, mitosis, meiosis and fertilisation
» DNA is a helical double-stranded molecule that occurs bound to proteins in chromosomes in the nucleus, and as unbound circular DNA in the cytosol of prokaryotes, and in the mitochondria and chloroplasts of eukaryotic cells
» variations in the genotype of offspring arise as a result of the processes of meiosis, including crossing over and random assortment of chromosomes, and fertilisation, as well as a result of mutations

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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2.1 THE CONTINUITY OF LIFE

All living things are made of cells and all cells originate from pre-existing cells. Each cell contains **genetic** information that codes for **traits** that are passed on to future generations. For life to continue, the processes of **cell division** must occur. Cell division is the splitting of a cell into new functioning cells. Organisms require cell division for growth, development, repair and reproduction. In biology, **heredity** is the study of the processes that are involved in transmitting genetic material to the next generation. For example, when black swans breed to produce a cygnet, the process of **meiosis** is required to create the cells that will fuse (during **fertilisation**) to form the cygnet's first cell. After meiosis and fertilisation, **mitosis** plays a major role in the growth of the cygnet.

Shutterstock.com/Jessica Dale

FIGURE 2.1 This cygnet has been formed by the process of cell division.

All individual organisms have a finite life span, but their species continue to exist. This is because some members of the species reproduce and pass on specific instructions embedded in their **DNA (deoxyribonucleic acid)**. DNA is a double-stranded helix with repeating units (building blocks) called **nucleotides**. A nucleotide is made up of three parts: a five-carbon sugar, a phosphate group and a **nitrogenous base**.

It is the DNA that determines the characteristics that define species. In all living things, DNA is the molecule that contains the instructions, written in a chemical code, for the production of proteins by the cell; the information it contains is sufficient for the making and maintaining of an organism. In addition, DNA is the genetic material that passes on this information to the next generation.

Key concept

DNA is a helical, double-stranded molecule that contains the building blocks of life. It determines the characteristics of all species and is passed down from generation to generation.

Living things that originate from one parent cell are said to be the product of **asexual reproduction**. In this process, the offspring are produced without fusion of **gametes**. It usually results in identical offspring that closely resemble their parent because they have only one source of inherited information.

Organisms that reproduce via **sexual reproduction** have two sources of hereditary material. Sexual reproduction is a process in which specialised male and female reproductive (sex) cells, called gametes, are produced, and then fuse to form a **zygote**. Fertilisation occurs when the two gametes join to form the zygote. Sexually reproducing organisms have a much greater potential for differences in characteristics between generations than asexually reproducing organisms.

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Chromosomes of eukaryotes

Eukaryotic cells are complex cells containing many membrane-bound **organelles**, including a nucleus. In eukaryotic cells, DNA is found in the **nucleus** bound to **histone** proteins. DNA is also found in the chloroplasts and mitochondria; however, this form is not bound to proteins. The combination of DNA and histone proteins found in the nucleus is called **chromatin**.

When the cell is not dividing, the chromatin is organised into a relatively loosely coiled form. As the cell prepares to divide, chromatin coils more tightly and becomes visible as **chromosomes**. During the process of cell division, the chromosomes appear in the nucleus as duplicated structures linked at a point called the **centromere**. Chromosomes are normally only visible under the microscope during cell division, and only then when stained (Figure 2.2).

Key concept

In eukaryotic cells, DNA is bound to histone proteins in the nucleus and is unbound in the mitochondria and chloroplasts.

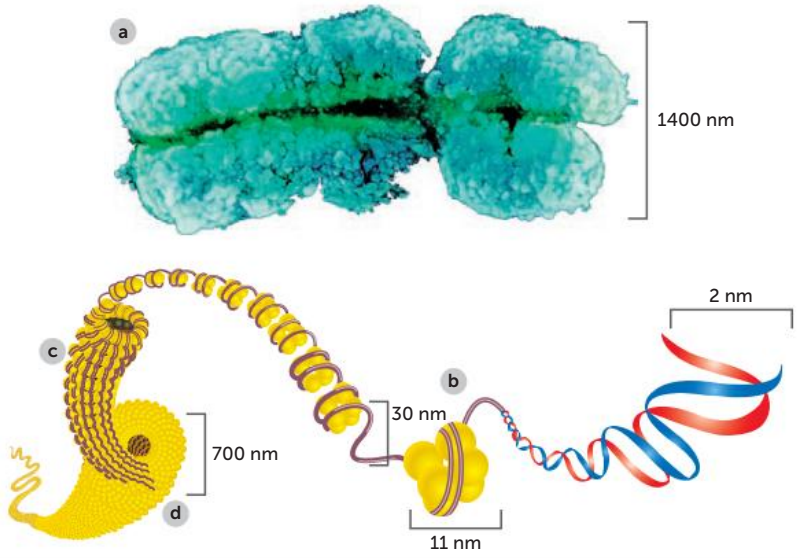

FIGURE 2.2 Levels of organisation of a human chromosome. **a** A tightly coiled and condensed human chromosome (only visible during cell division and when stained). **b** A nucleosome, consisting of a section of a DNA molecule looped twice around a core of eight histone proteins. **c** Interacting proteins package loops of coiled DNA and protein, called chromatin, which is organised as a cylindrical fibre. **d** Chromatin is further condensed to make chromosomes.

Key concept

A chromosome is made up of a double-stranded DNA molecule wrapped around its associated histone proteins.

In many eukaryotes, there are pairs of chromosomes, with one chromosome of each pair being inherited from each parent. Examination of a prepared microscope slide of stained cells in the process of nuclear division reveals a jumbled cluster of chromosomes that differ in size and shape. The chromosomes visible in a photographic image of the microscope slide can be arranged into matched and ordered pairs to create a **karyotype**, the standard graphical form used to display and analyse chromosomes (Figure 2.3, page 28). Most of the chromosomes are ordered according to length, from largest to smallest. Each species of organism typically has a particular number of chromosomes in each cell.

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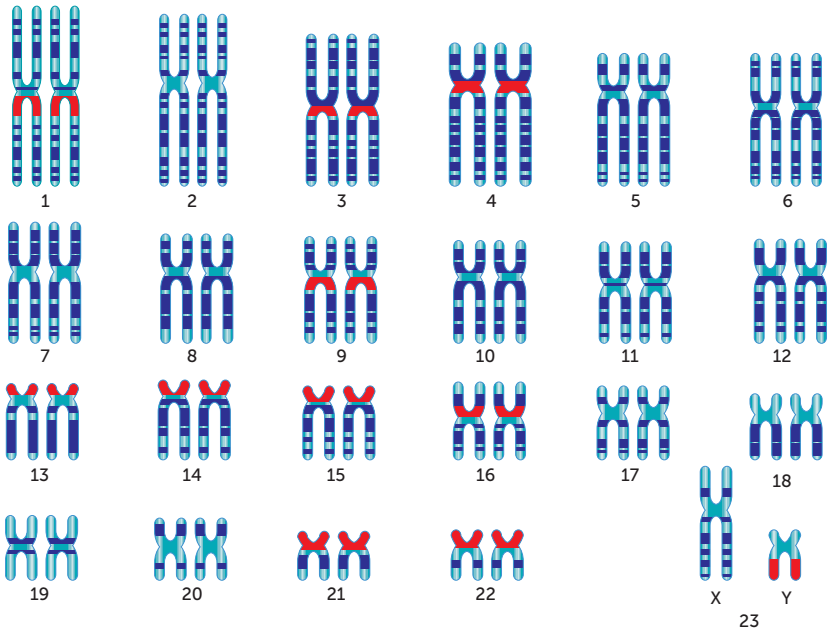

FIGURE 2.3 A male human karyotype showing 23 pairs of chromosomes

The nucleus of each **somatic cell** (body cell) of a human contains 46 chromosomes, which form 23 pairs, of which 22 are matched **homologous chromosomes** (Figure 2.4). The nucleus of a Tasmanian devil somatic cell contains 14 chromosomes, which form 7 pairs, of which 6 are homologous. The chromosomes are recognisable individually by their size, position of centromere and banding pattern. The bands on the chromosomes correspond to large groups of **genes**.

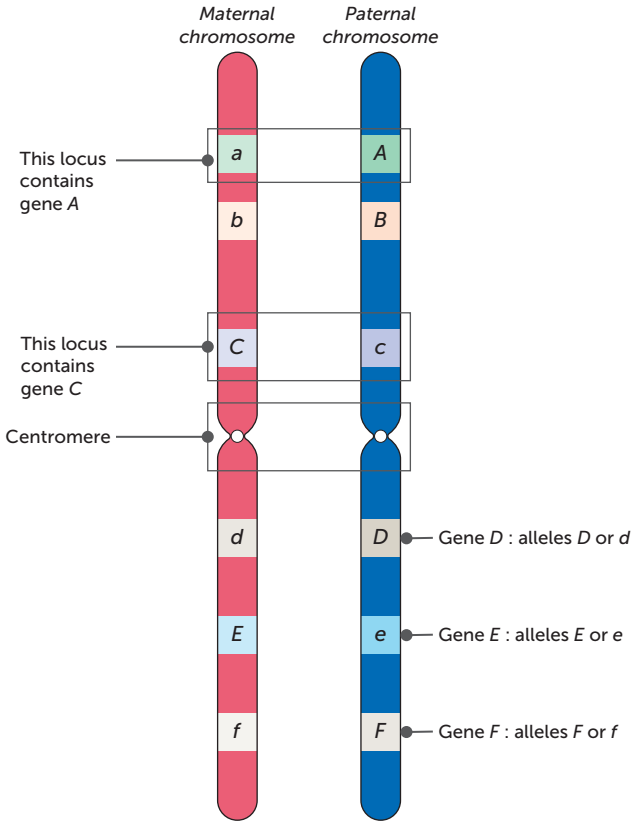

FIGURE 2.4 Stylised representation of a pair of chromosomes. In the somatic cells of diploid organisms, one of each pair of chromosomes comes from the male parent and the other from the female parent.

The **paternal chromosome** in each pair of chromosomes comes from the male parent (via the male gamete), and the **maternal chromosome** comes from the female parent (via the female gamete).

The matched homologous pairs of chromosomes are also called **autosomes**. In humans the 23rd pair of chromosomes is matched in females (XX) and unmatched in males (XY). As such, this pair of chromosomes in males is called a **heterosome**. Because the X and Y chromosomes determine the sex of an individual, they are also referred to as **sex chromosomes**. The 7th pair of chromosomes in a Tasmanian devil's karyotype are the sex chromosomes.

Early in an embryo's development, its **germline cells** specialise into male or female gametes through the process of **differentiation**.

The number of chromosomes in each somatic cell is called the **diploid** number and is represented as $2n$. As chromosomes occur in pairs, n stands for the number of chromosomes in one complete set found in one gamete, and the number of pairs that the particular species has in each of its other cells. This is called the **haploid** number. A human somatic cell has 23 pairs, so its diploid number is $2n = 46$ and its haploid number is $n = 23$.

Along the length of each DNA molecule, there are regions of DNA (genes) that code for specific proteins. These proteins determine the particular characteristics or traits of the organism. The location of a specific gene on a chromosome is referred to as its **locus (plural loci)**. In homologous chromosomes, the corresponding gene is found at the same locus on each of the pair of chromosomes. Alternative forms of the same gene are called **alleles**. Alleles are versions of the same gene with slight differences. Sometimes there is just one single difference in the genetic sequence, but it may be enough to cause large variation in the functioning of the gene. For example, a change in the genetic sequence in a single gene causes juvenile hereditary cataracts (JHC) in French bulldogs that have two copies of the changed allele. A normal, diploid organism has two of each gene in every somatic cell with a nucleus. One is on the maternal chromosome and the other on the paternal chromosome in a particular homologous pair, so one gene comes from each parent. For the majority of genes, there is only one allele (or gene variant), but it is the different alleles that exist for some genes that give individuals distinct traits.

Chromosomes of prokaryotes

Membrane-bound organelles, such as a nucleus, are not present in the single-celled organisms known as **prokaryotes**. The DNA within these cells generally forms a single circular chromosome that lies in direct contact with the cytosol (intracellular fluid) (Figure 2.5, page 30). The chromosome is often joined to the cell membrane at a single point. Although not contained by an internal membrane, the chromosome can be in a distinct region of the cell called a **nucleoid**. Additional small rings of DNA, called **plasmids**, may also be present in the cytosol. Non-essential proteins are commonly encoded on these plasmids. Plasmids can replicate independently of the main chromosome. They have become important tools in genetic engineering, because they can be easily transferred from one bacterium to another and replicate rapidly.

Key concept

In prokaryotes, DNA is found in the cytosol as unbound circular DNA.

Eukaryotic chromosomes are linear, with longer lengths of DNA than is present in prokaryotes, and they need to condense into a small volume. A number of proteins work together to fold and condense the DNA into chromatin. Prior to dividing, chromatin is condensed even further by supercoiling to form chromosomes. Prokaryotic cells have less DNA because they are generally haploid (i.e. only contain one copy of each gene) and they contain less repetitive non-coding DNA.

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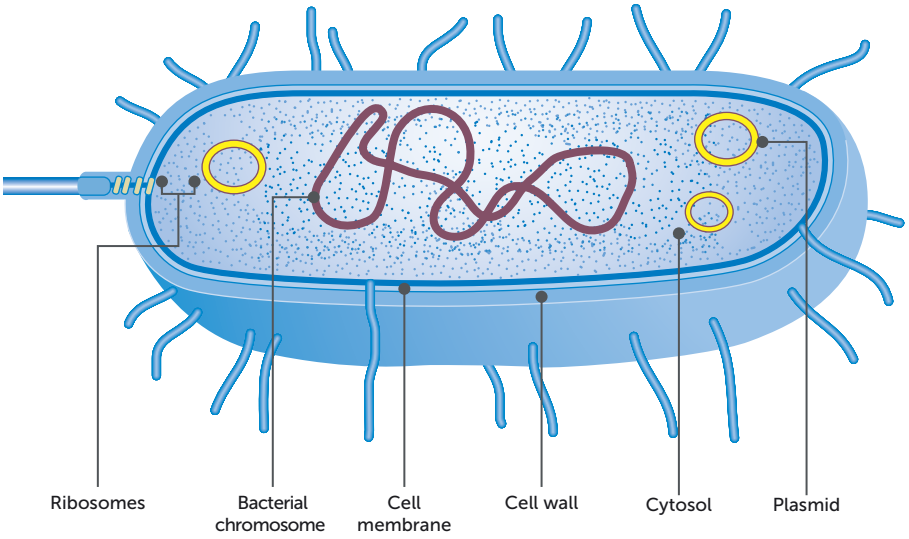

FIGURE 2.5 DNA in a prokaryotic cell

Just as with many other strategies in nature, there are exceptions. Not all bacteria have a single circular chromosome. There are some bacteria with more than one circular chromosome. Other bacteria have linear chromosomes and linear plasmids. Another notable difference between the chromosomes in prokaryotes and those in eukaryotes is the presence of histones. Most prokaryotes do not have histones (with the exception of some species in the domain Archaea).

TABLE 2.1 Differences between typical prokaryotic and eukaryotic cells

FACTOR	PROKARYOTIC CELL	EUKARYOTIC CELL
Typical diameter	1–5 micrometres	10–100 micrometres
Location of DNA	In cytosol (in nucleoid)	In nucleus, mitochondria and chloroplasts
Membrane-bound organelles	No membrane-bound organelles	Membrane-bound organelles, including a nucleus
Ribosomes	Yes, they float freely in the cytosol	Yes, they can float freely in the cytosol or be attached to the endoplasmic reticulum
Chromosome(s)	A single circular DNA strand (typically, without histones)	DNA is wrapped around proteins (called histones), creating units called nucleosomes. This loosely coiled form of DNA and protein is called chromatin. The chromatin coils more tightly ('condenses') to form chromosomes in preparation for mitosis or meiosis.

Question set 2.1

REMEMBERING

1 Define:
   - **a** cell division
   - **b** sexual reproduction
   - **c** asexual reproduction.

2 Describe the process of constructing a karyotype.

3 Draw a pair of chromosomes and label the centromere, genes and loci.

UNDERSTANDING

4 Explain the difference between haploid and diploid numbers of chromosomes, using an example.

5 Explain why fertilisation only occurs during sexual reproduction.
6 Discuss the relationship between genes and traits.
7 Distinguish between prokaryotic cells and eukaryotic cells.

ANALYSING

8 Analyse the information about eukaryotic and prokaryotic cells, and describe three of the similarities.

2.2 CELL DIVISION

Within an organism, eukaryotic cells pass on their instructions for growth and development from one generation of cells to the next during cell division. To complete the process of cell division, both nuclear division (mitosis or meiosis) and cytoplasmic division (**cytokinesis**) must occur. Mitosis is a type of nuclear division occurring in somatic cells that maintains the parental diploid number of chromosomes in the daughter cells; it is the basis of both bodily growth and asexual reproduction in many eukaryotic species.

The terms 'parent' and 'daughter' cells are used for communication purposes to help distinguish the original cell from the newly formed cells.

Under normal circumstances, cell division takes place through an orderly process. Mitosis and cytokinesis result in the formation of two diploid daughter cells, which each contain identical sets of chromosomes.

Meiosis is the form of eukaryotic cell division concerned with the production of gametes (sex cells) in sexually reproducing organisms. Meiosis, a type of cellular division involving one cycle of DNA replication and two rounds of cell division, results in the production of four haploid daughter cells from each original diploid parent cell.

At fertilisation, two haploid gametes, a male and a female, combine to form a diploid zygote.

Key concept

Eukaryotic cell division involves a number of phases, including nuclear division (mitosis or meiosis) and cytoplasmic division (cytokinesis). The processes of mitosis and meiosis allow for the replication and transfer of genetic material to the next generation.

The cell cycle

The sequence of events from one cell division to another is called the **cell cycle** (Figure 2.6, page 32). The cell cycle is the ordered sequence of events in the life of a cell. It begins when the cell is formed from its parent cell and is completed with its own division. Even though we describe this cycle as taking place in phases, in reality it is usually a continuous process. The cell division stage, in which nuclear division and cytokinesis occur (known as M phase, or mitotic phase), is only a small part of the cycle. The stage between cell divisions is called **interphase**, and it incorporates a period of metabolic activity and growth (G1 phase, or first 'gap' phase), duplication of the chromosomes (DNA replication) and of the **centrosomes** (S phase, or 'synthesis' phase), and further growth and reproduction of organelles as the cell prepares to divide (G2 phase). A further phase, G0, can also be seen in Figure 2.6. Cells in G0 are in the non-proliferating state – they are undergoing an extended G1 but are not preparing to replicate their DNA and divide. Cells in G0 have withdrawn from the active cell cycle and can only re-enter the cell cycle under certain circumstances.

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Control of the Cell Cycle Game
As a 'cell division supervisor' inside the cell nucleus, you are to steer the cell division process to make sure everything happens in the right order.

The length of the cell cycle varies from cell to cell, and not all cells divide. For example, most specialised cells, such as nerve cells and retinal cells in adult humans, do not divide. On the other hand, during developmental growth and in areas of high wear, cells divide frequently. Cells can divide to create new organs or tissues. For example, cells in a growing root tip may divide every 20–24 hours. Cells in high-wear areas, such as in the skin or in the lining of the mouth or gut, divide to replace the dead cells 'sloughed off' due to mechanical disturbance, or in response to new cells growing below.

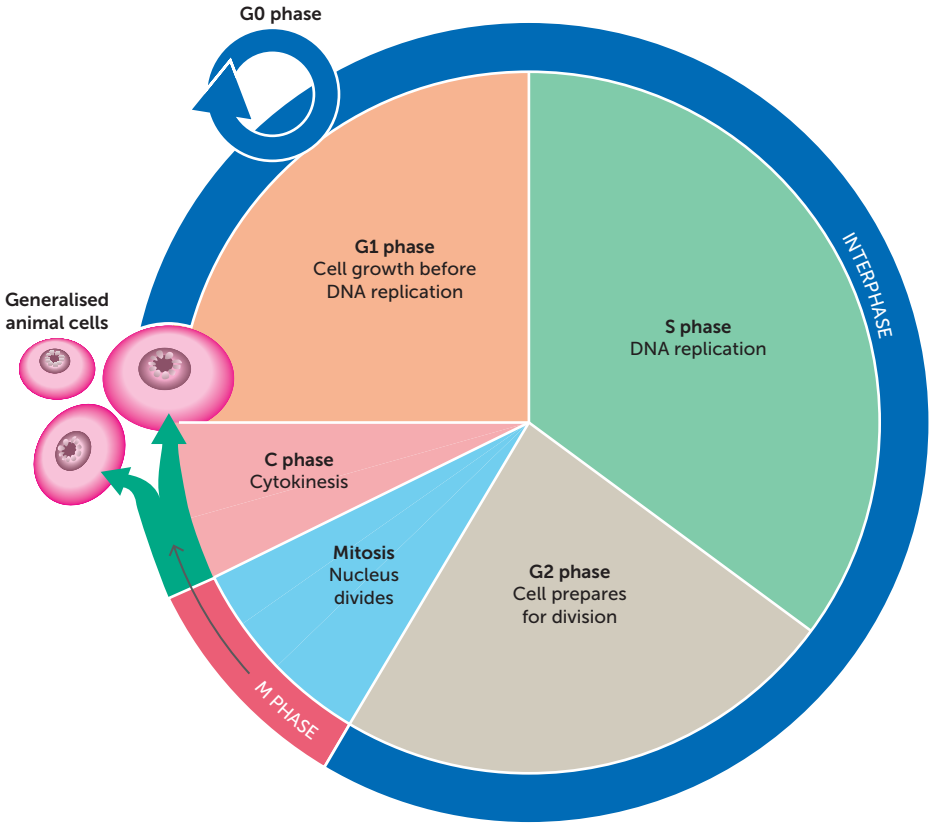

FIGURE 2.6 The life cycle of a cell. Most cells spend the majority of their time in interphase.

Question set 2.2a

REMEMBERING

1 Define:
   a interphase
   b mitosis
   c meiosis.
2 List and define the six phases of the cell cycle.

UNDERSTANDING

3 Explain why the terms 'parent' cell and 'daughter' cell are used.
4 Distinguish between mitosis and cytokinesis.

ANALYSING

5 After analysing the cell cycle, draw and label a cell cycle of your own.

Mitosis in eukaryotic cells

Prior to any cell division, a doubling of the genetic material needs to take place. This happens during interphase (Figure 2.6). Chromosomes are not visible during interphase and cannot be clearly distinguished under a light microscope or an electron microscope. As the cell leaves interphase and begins mitosis, the chromatin threads get shorter and thicker, becoming visible under a light microscope. The centrosome, which contains two **centrioles**, becomes visible and is duplicated during the S phase in many cells. The centrosomes produce the spindle fibres during mitosis and meiosis. They also facilitate correct separation of the chromosomes into the daughter cells.

Also during the S phase, the chromatin duplicates and the cell doubles its amount of genetic material. Chromosomes form from the chromatin during the first phase of cell division in the cell cycle, known as prophase. The DNA and histones condense, and each chromosome becomes visible for the first time, appearing as an 'X' shape. The 'X' is made up of two **chromatids** attached to each other over a small region of their DNA called the centromere (Figure 2.7). Even though each chromosome consists of two chromatids, it is still considered to be one chromosome. The centromere is important for the attachment of chromosomes to spindle fibres. Spindle fibres are generated by the centrioles, which are the two small organelles inside each centrosome. Centrioles use the spindle fibres to move the sister chromatids to opposite ends of the cell during cell division.

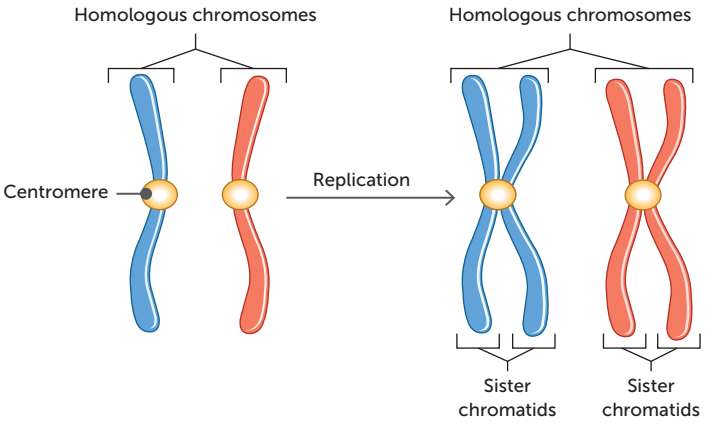

FIGURE 2.7 Homologous chromosomes at two different stages of the cell cycle

Mitosis is a relatively short part of the cell cycle, and is broken down into four phases: prophase, metaphase, anaphase and telophase. The phases of mitosis are described in detail in Table 2.2 (page 34). At the conclusion of mitosis, there is a diploid number of chromosomes in the daughter cells, but they have half the genetic material of the parent cell in prophase. It is important to remember that one chromosome can consist of either one DNA double helix or two DNA double helices connected by a centromere. This helps clarify why the chromosomes of a parent cell at the start of mitosis look different from those of a daughter cell at the end of mitosis, even though there is a diploid number of chromosomes in both of them.

Following mitosis, cytokinesis occurs, which forms two separate, diploid daughter cells. The daughter cells produced by mitosis are usually identical, and therefore very little variation in offspring arises during mitosis. Mitosis is nuclear division, during which the genetic material in the parent cell is replicated, and cytokinesis is the division and separation of the cytoplasm to create the new daughter cells.

Key concept

Identical daughter cells are produced as a result of mitosis; in other words, variation between daughter cells is limited in mitosis.

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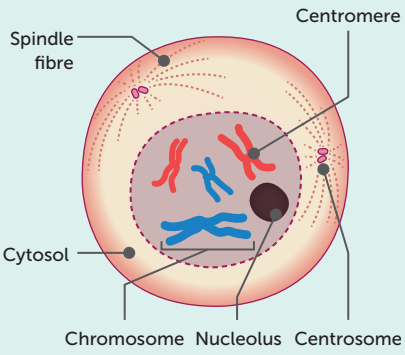
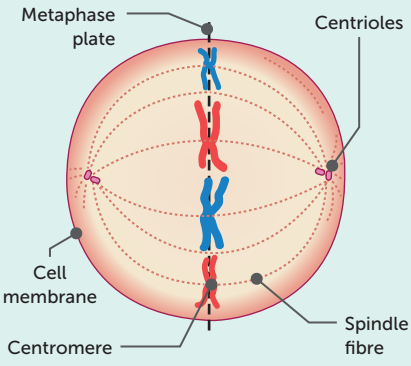
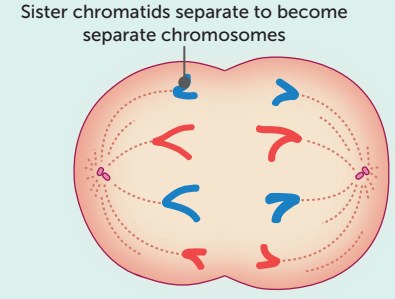
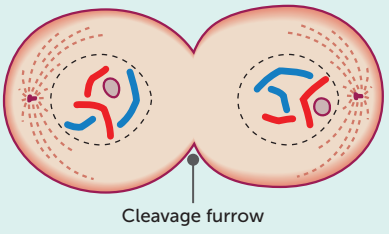
TABLE 2.2 Mitosis – four main phases

PHASE	LABELLED ILLUSTRATION
Prophase **1** Chromatin threads condense to form chromosomes and become visible under the microscope. **2** Chromosomes consist of two sister chromatids held together by a centromere. **3** The nuclear membrane disintegrates and the **nucleolus** (organelle inside the nucleus where ribosomes are assembled) disappears. **4** The mitotic spindle begins to form and is completed by the end of prophase. The spindle fibres attach to each chromosome at its centromere. **5** The two centrosomes (each containing two centrioles) move towards opposite poles of the cell.	 **FIGURE 2.8** Prophase
Metaphase **1** The chromosomes move to the centre of the cell and line up along the equator of the cell. The equator is also referred to as the metaphase plate. **2** The centromeres of the chromosomes are aligned on the equator. **3** The centrioles are located at opposite poles of the cell.	 **FIGURE 2.9** Metaphase
Anaphase **1** The spindle microtubules shorten and pull on the centromeres; the sister chromatids separate. **2** The spindle microtubules pull the sister chromatids to opposite poles of the cell. **3** The centromere, being attached to the microtubules (spindle fibres), is the first part of each chromosome to be pulled towards the poles. The 'arms' of each chromatid follow as they are pulled along by the centromere. **4** At the end of this phase, each pole has a complete identical set of maternal and paternal chromosomes. (The genetic material doubled during the S phase, before cell division started, so the amount of DNA at each pole is the same as that of the interphase parent in G1.) **5** The sister chromatids are now referred to as chromosomes.	 **FIGURE 2.10** Anaphase
Telophase **1** Chromosomes decondense to form chromatin, at which time they can no longer be seen under the microscope. **2** Two new nuclear membranes (also known as nuclear envelopes) form, one for each new daughter cell. **3** Nucleoli reappear and the spindle apparatus disappears. **4** The cell elongates and a cleavage furrow forms to become ready for cytokinesis.	 **FIGURE 2.11** Telophase

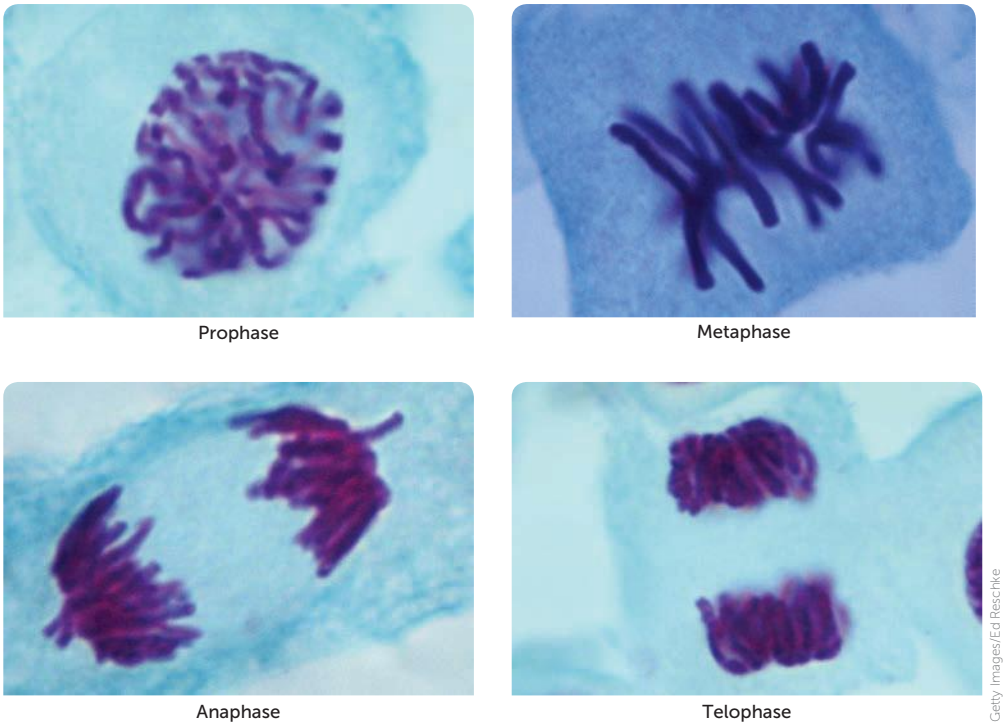


FIGURE 2.12 These micrographs show prophase, metaphase, anaphase and telophase in onion root tip cells.

Cytokinesis in eukaryotic cells

Plant cells

The cytoplasm of plant cells divides with the formation of a structure called a **cell plate**. Figure 2.13 shows how parts of the cell wall fuse with parts of the spindle, forming the cell plate. Cellulose is deposited at this site, forming a wall that divides the parent cell into two daughter cells, each one with a cell membrane.

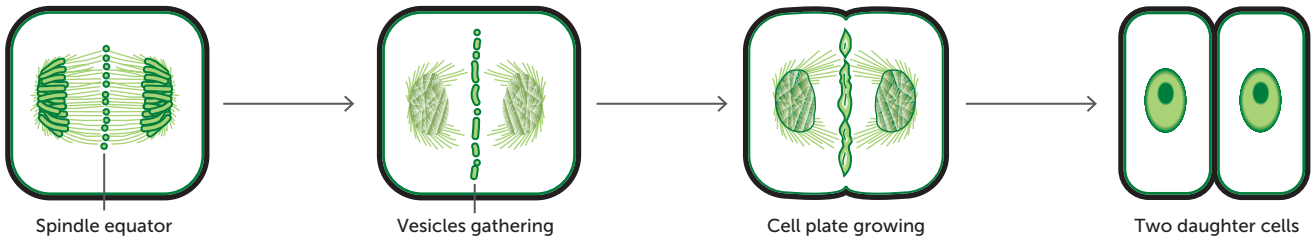


FIGURE 2.13 Cytokinesis in a plant cell involves the formation of a cell plate; cellulose is deposited on the cell plate to complete the cell walls of the two new daughter cells.

Animal cells

Animal cells do not have a cell wall, and so cytokinesis in animal cells does not require the formation of a cell plate. In animal cells, the cytoplasm divides by a process known as **cleavage**. The cell membrane around the middle of the cell draws together to form a **cleavage furrow**. The cleavage furrow continues to develop until the cell membrane eventually meets at a point, and the cell is then cleaved, or split, resulting in two new daughter cells (Figure 2.14).

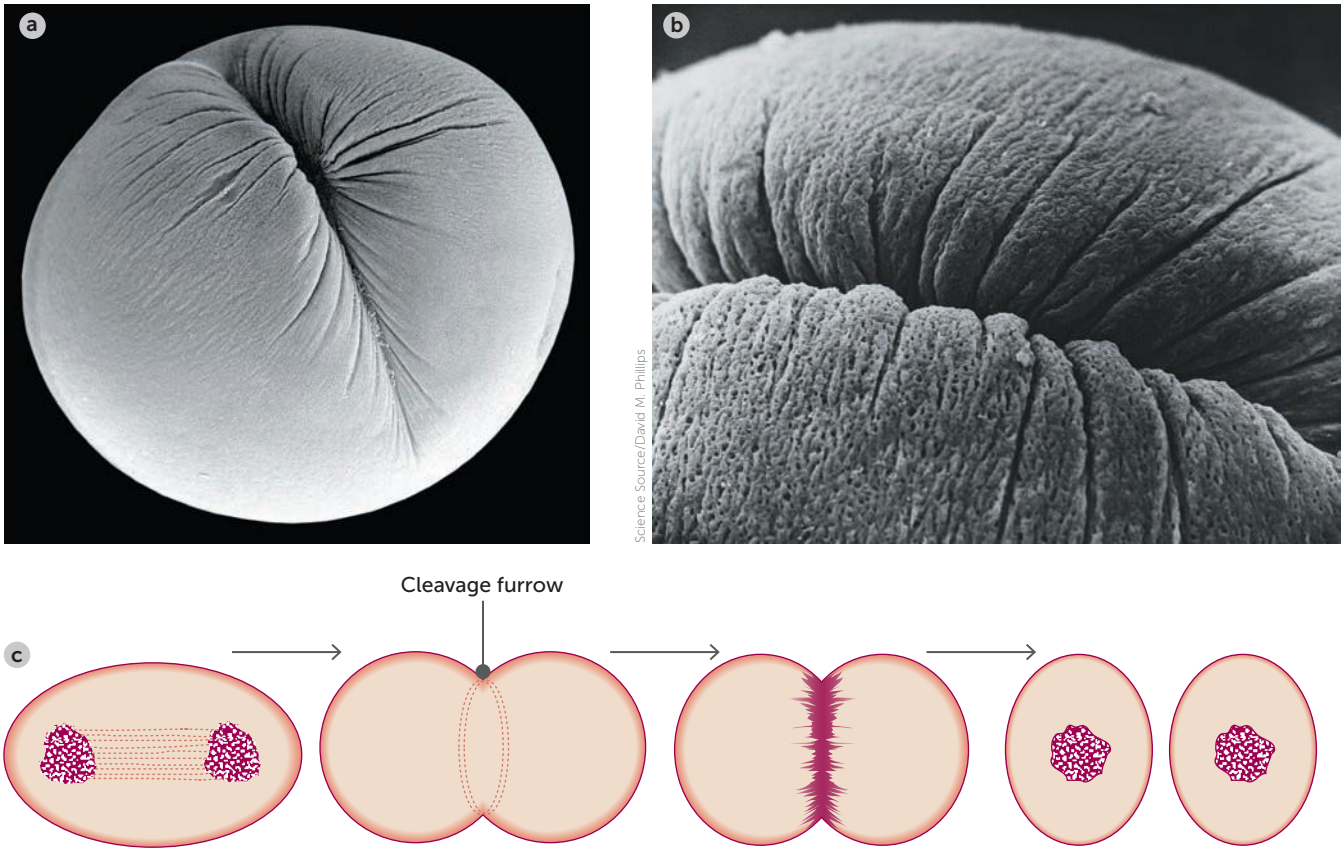


FIGURE 2.14 a Micrographs of an animal cell during cytokinesis, showing the cleavage furrow from a distance and **b** close up. **c** After formation of a cleavage furrow, the cell divides to produce two new daughter cells.

Mitosis and meiosis animations
Watch this animation that explains mitosis and meiosis side by side.

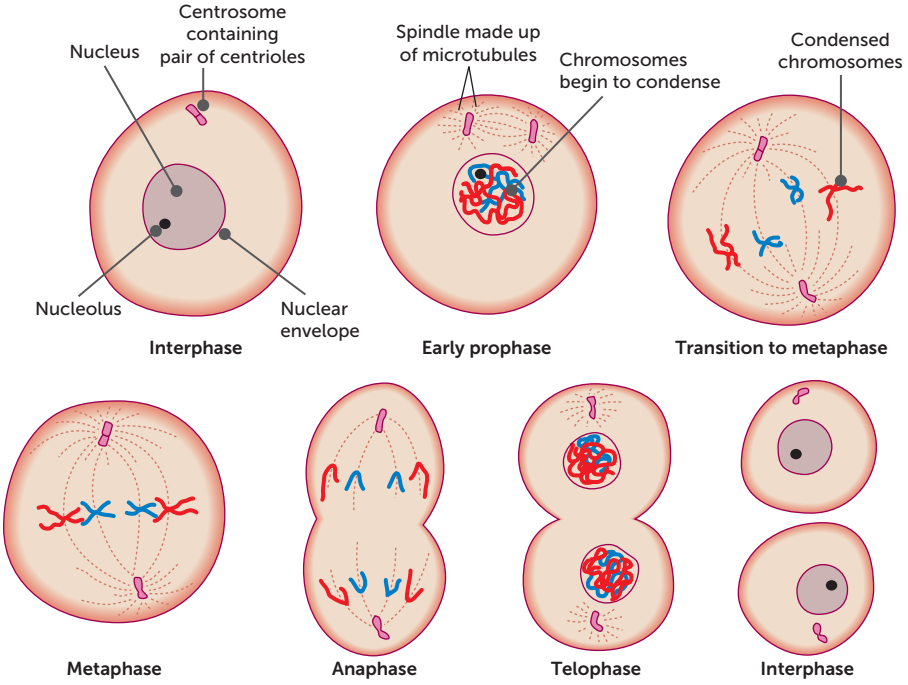


FIGURE 2.15 Mitosis is a continuous process.

Question set 2.2b

REMEMBERING

1. Name the four phases of mitosis.
2. Draw and label a chromosome prior to the S phase and after the S phase in the cell cycle.
3. What is one major difference between cytokinesis in plant cells and animal cells?

UNDERSTANDING

4. Draw a labelled diagram to show your understanding of the following terms:
   - a maternal and paternal homologous chromosomes
   - b diploid
   - c spindle microtubules (spindle fibres)
   - d centromere.
5. Discuss whether homologous chromosomes have the same number of genes or identical genes.
6. Compare the diploid number of chromosomes at the beginning of mitosis in the parent cell with the diploid number of chromosomes at the end of mitosis in the daughter cells.

APPLYING

7. Construct a diagram showing the four phases of mitosis and cytokinesis using three pairs of homologous chromosomes.

Marsupial chromosomics: bridging the gap between genomes and chromosomes

CASE STUDY

The number and general appearance of a set of chromosomes within the nucleus of a typical human body cell was first published in 1956. The process of pairing and ordering the chromosomes of an organism is called karyotyping. A karyotype provides a snapshot of an organism's **genome** (all of the genetic material contained in an organism or a cell). One of the first organisms that had its chromosomes ordered and paired was Thale cress, a small flowering plant that has five chromosomes.

Over time, parts of chromosomes can change. Researchers at CSIRO and the University of Canberra have spent time investigating these changes in marsupial genomes. The first marsupial genome was sequenced only a decade ago, but since then advances in biotechnology have made the sequencing of many more marsupials possible. Comparing chromosomes of different marsupial genomes can help scientists start to unravel mysteries relating to speciation, adaptation and survival.

Evolution of marsupial genomes
What do genomes have to say about marsupial history?

Questions

1. Name one of the first organisms to have its chromosomes' DNA sequenced.
2. Describe the relationship between a karyotype and a genome.
3. State one reason why it may take longer to sequence a marsupial's genome, compared with that of a plant such as Thale cress.

Alamy Stock Photo/Bill Bachman

FIGURE 2.16 Genomic studies of marsupials, such as this tiny western pygmy possum, are being used to find their evolutionary relationships.

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Binary fission

Cell reproduction is more complex in eukaryotes than in prokaryotes. As prokaryotes lack a nucleus and have only a single chromosome with no centromere, they cannot be properly said to undergo mitosis. They reproduce by **binary fission**. Binary fission can be defined as a process of asexual reproduction whereby a prokaryotic cell divides into two identical daughter cells. The process includes DNA replication, chromosome segregation and cytokinesis. As in mitosis, binary fission produces daughter cells with the same number of chromosomes as the parental cell.

There is limited variation in prokaryotic populations other than that due to mutation. However, binary fission is a process that happens relatively fast compared with other cell division processes, which means the mutation rate is much higher.

Prokaryotic bacterial cells replicate their single DNA strand, signalling the beginning of binary fission. Following replication, each DNA copy attaches to a different part of the cell membrane. When the cell begins to lengthen during cell division, the replicate (copy) and original chromosomes are separated. A wall forms across the cell and divides it into two cells of identical genetic composition.

A process similar to binary fission occurs in eukaryotic cells when mitochondria and chloroplasts divide to form new organelles. They divide independently of the nuclear DNA but segregate evenly into the two daughter cells during cytokinesis.

Binary fission can be summarised into six steps, but it is helpful to note that, like mitosis and meiosis, binary fission is actually a continuous process, not one that stops and starts.

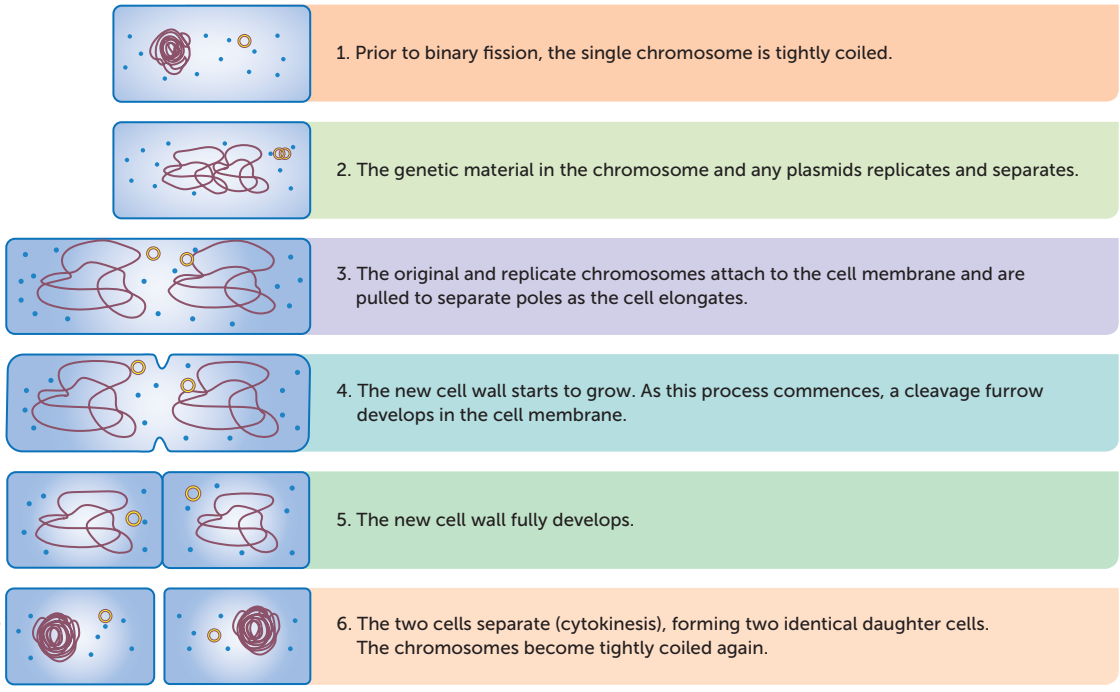

FIGURE 2.17 Prokaryotic cells reproduce by binary fission.

Question set 2.2c

REMEMBERING

1 Place the following terms in order for the main processes of binary fission.

Cleavage furrow develops
Chromosome replicates
New cell wall is completed
Cytokinesis
Cell elongates

UNDERSTANDING

2 Explain what causes variation in prokaryotes and why this may occur at a higher rate than in eukaryotes.

3 Draw a labelled diagram to show the sequence of events of binary fission. You may like to create a binary fission poster.

Meiosis in eukaryotic cells

Meiosis occurs in specialised organs in sexually reproducing animals and plants. It results in the production of gametes, also known as sex cells. In animals, male and female gametes are called sperm and eggs (ova). In many plants, male and female gametes are found within pollen and ovules. In some plants, spores are produced that grow into haploid plants (gametophytes).

Meiosis is a unique type of cell division in that the daughter cells produced have only half the number of chromosomes of the parent cell. This prevents the doubling up of the diploid number of chromosomes at fertilisation. In meiosis, two divisions of the nucleus of the parent cell take place. In the first division, the chromosomes of each pair separate and go to either end of the cell. In the second division, the chromatids of each chromosome separate from each other. Four haploid gametes are thus produced, each carrying half the original number of chromosomes. As the number of chromosomes is reduced by half, meiosis is called a reduction division.

The reason why there are pairs of chromosomes in somatic cells, two of each chromosome type, is because one is from the father and one is from the mother. The pair are often referred to as paternal and maternal homologous chromosomes. As mentioned previously, scientists use the letter *n* to represent the number of chromosomes in one gamete; it is also the number of chromosome pairs in an organism. During meiosis, the chromosomes of each homologous pair separate to each gamete at random, contributing to the variation in offspring.

The phases of meiosis represent the continuous process by which a diploid parent cell gives rise to four haploid, non-identical daughter cells. Prior to meiosis, duplication of DNA occurs. The amount of genetic material doubles, without changing the number of chromosomes. This results in each chromosome taking the form of two identical sister chromatids joined to each other by a centromere. In meiosis, in order to produce four cells, each with half the original number of chromosomes, a second division occurs.

During meiosis I, the homologous chromosomes pair up and physically connect (each pair is now called a **bivalent**) in a process called **synapsis**. During the first step of meiosis, prophase I, there is an exchange of genetic material between maternal and paternal homologous chromosomes (i.e. between non-sister chromatids of the homologous chromosomes). This process is called **crossing over**. Crossing over allows DNA from the person's maternal chromosome to swap with DNA from the paternal chromosome. At the end of meiosis, the chromosomes in the gametes are a recombination of maternal and paternal genes. In eukaryotic cells, crossing over, independent assortment and random fertilisation contribute to variation in a population. The independent assortment occurs due to random orientation of the maternal and paternal homologous chromosomes during metaphase I, which results in their random assortment into the gametes at the conclusion of meiosis.

Cells alive
Watch the meiosis animation and determine whether the phases match those found in Table 2.3 (page 40).

Key concept

The crossing over and random assortment of chromosomes during meiosis contribute to variations in offspring.

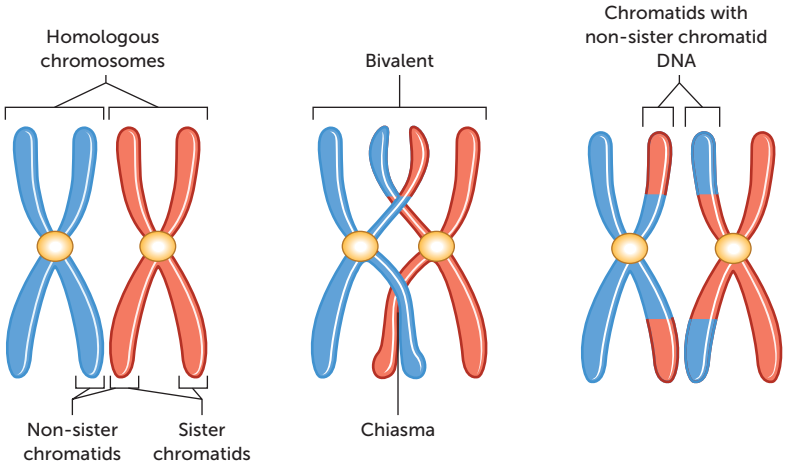

FIGURE 2.18 Crossing over

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TABLE 2.3 Phases of meiosis

PHASE	LABELLED ILLUSTRATION
Prophase I **1** Chromatin threads condense to form chromosomes and become visible under the microscope. **2** Maternal and paternal homologous chromosomes are attracted to each other and pair up (synapsis); crossing over occurs. **3** Each chromosome consists of two sister chromatids held together by a centromere. **4** The nuclear membrane disintegrates and the nucleolus disappears. **5** The meiotic spindle begins to form and attaches to chromosomes at the centromeres. **6** The centrosomes move to opposite poles of the cell.	
Metaphase I **1** The maternal and paternal homologous chromosomes line up along the metaphase plate (cell's equator) in pairs. **2** The lining up of the homologous chromosomes in metaphase I is called independent assortment because each pair is lined up on one side or the other, independent of every other pair. This results in a random assortment (random combination) of chromosomes in the four daughter cells. **3** The spindle fibres are attached to centromeres.	
Anaphase I **1** The spindle fibres shorten, pulling on the centromere of each chromosome. **2** One member of each pair of homologous chromosomes moves to each end of the cell. A random combination of maternal and paternal chromosomes are dragged to each pole.	
Telophase I **1** New nuclear membranes form and the chromosomes uncoil. **2** The spindle fibres disintegrate.	
Cytokinesis I: separation of the cytoplasm The cell splits into two cells. The daughter cells are considered haploid because they contain only one chromosome from each pair of homologous chromosomes. No further DNA replication occurs.	
Prophase II **1** Chromatin condenses to form visible chromosomes again. **2** New spindle fibres are produced. **3** The nuclear membrane disintegrates.	
Metaphase II **1** Individual chromosomes line up single file along the equator in random order. **2** The spindle fibres attach to the sister chromatids at the centromeres.	
Anaphase II **1** The centromeres of each chromosome disconnect, allowing the sister chromatids to separate. **2** The spindle fibres shorten and individual sister chromatids move to opposite poles of the cell. **3** In animal cells, the cell membrane pinches inwards to form a cleavage, whereas in plant cells new cell wall plates form.	
Telophase II **1** Chromosomes unwind, loosen and reform chromatin. **2** Four new nuclear membranes form around the nuclei, one in each new daughter cell.	
Cytokinesis II: separation of the cytoplasm The cells separate into four new non-identical, haploid daughter cells.	

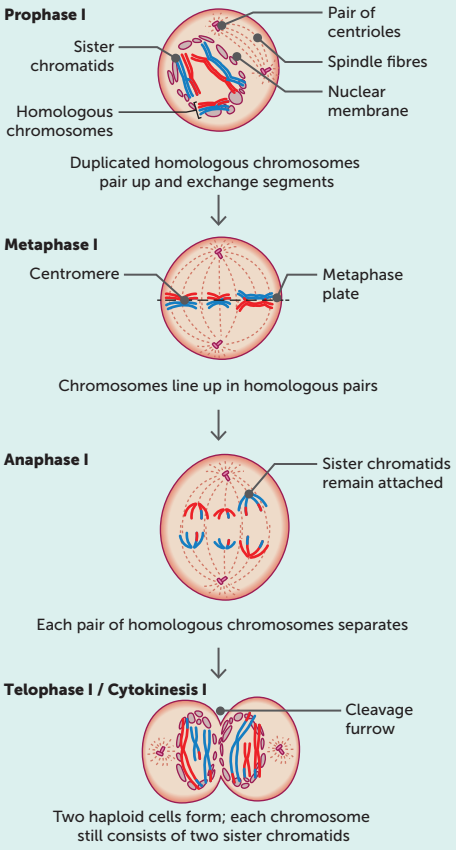

FIGURE 2.19 Meiosis I

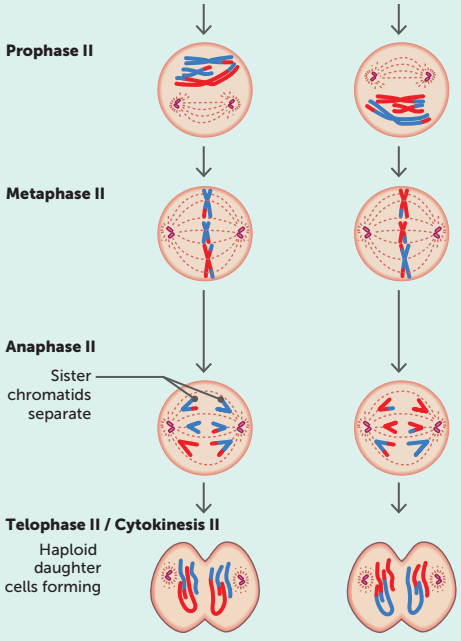

FIGURE 2.20 Meiosis II

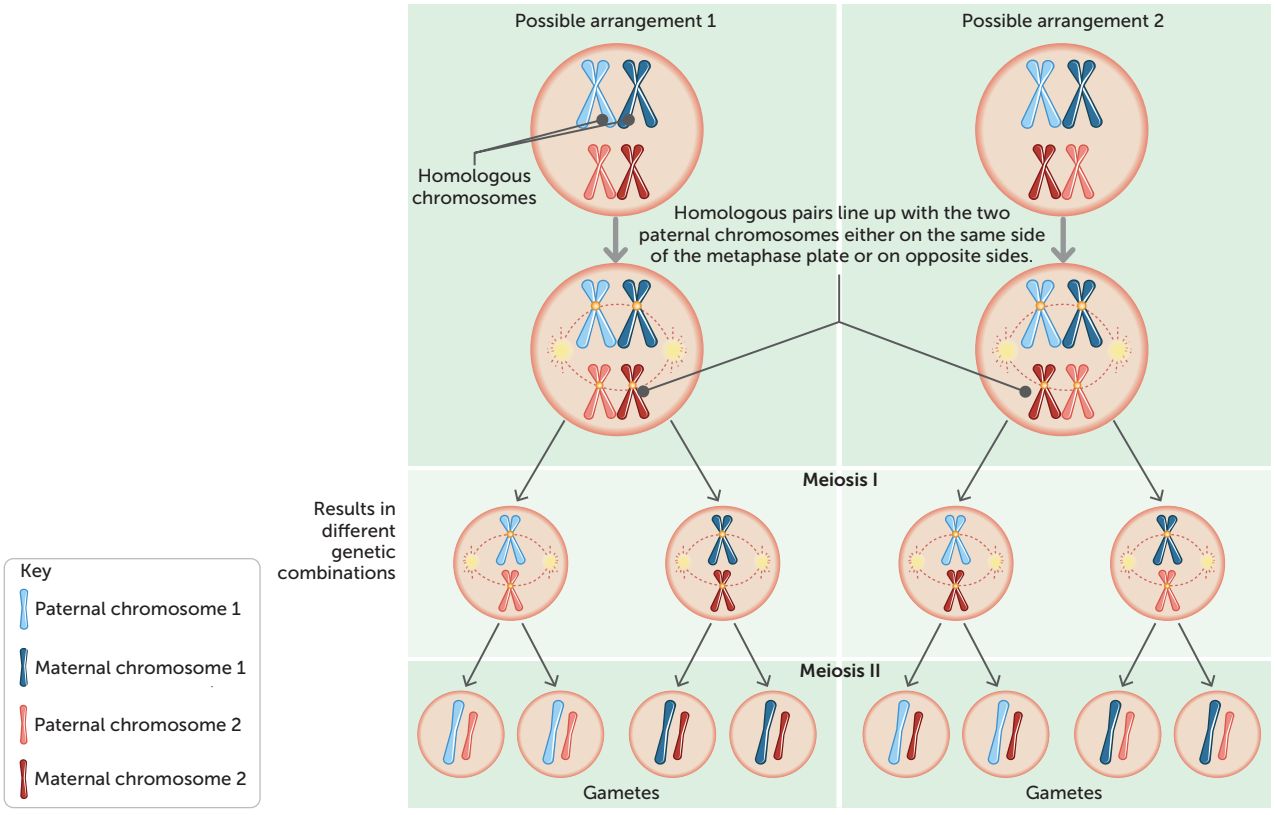

FIGURE 2.21 Independent assortment during metaphase I. Note: Mendel's law of independent assortment states that when gametes are formed, the assortment of one pair of chromosomes/alleles between the daughter cells is independent of that of another pair of alleles.

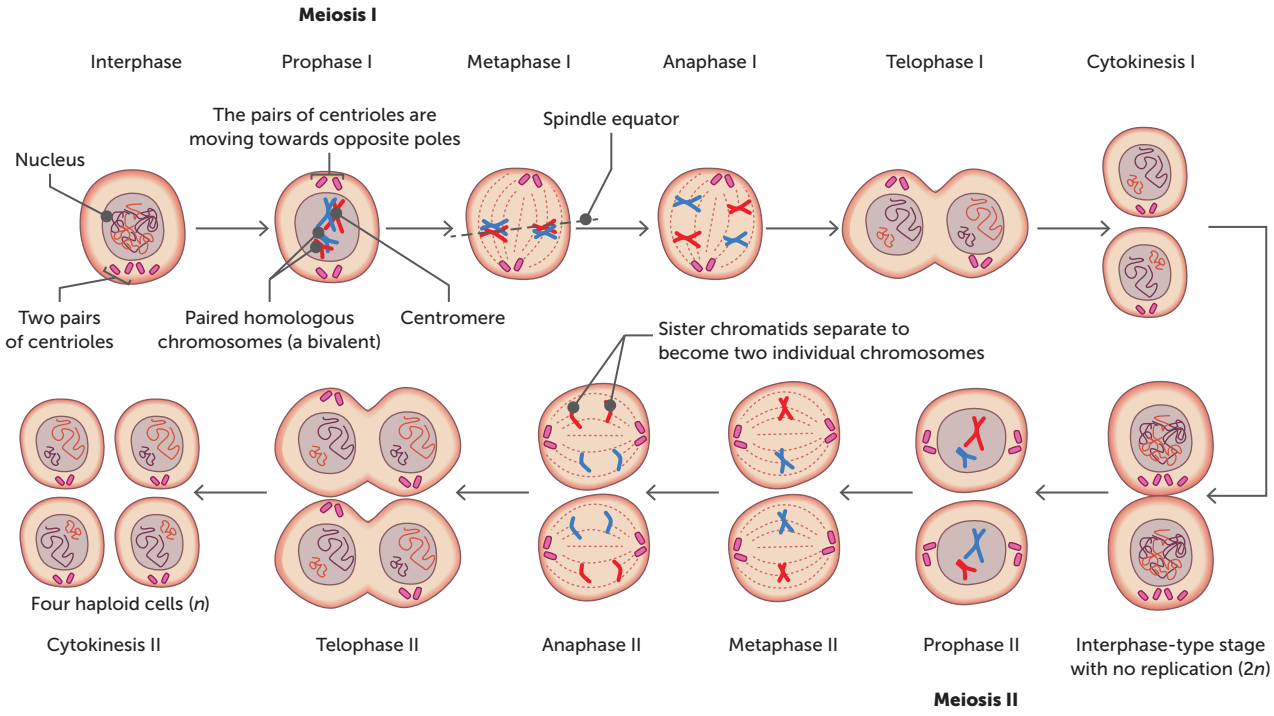

FIGURE 2.22 The stages of meiosis

TABLE 2.4 Comparison of mitosis and meiosis

FEATURES	MITOSIS	MEIOSIS
Function	Nuclear and cellular division for growth, repair and replacement of tissues.	Nuclear and cellular division for producing gametes.
Number of cell divisions	One	Two
Number of chromosomes in daughter cells	Each of the two identical daughter cells contains the diploid number of chromosomes (2*n*).	Each of the four, non-identical daughter cells contains the haploid number of chromosomes (*n*).
Variation	New cells or offspring produced by this kind of reproduction do not show variation between them unless there are environmental influences or mutations; they are genetically identical to one another (i.e. clones).	Offspring produced show variation between them due to crossing over in prophase I and independent assortment in metaphase I.
Diversity	Diversity of offspring does not increase.	Diversity of offspring is increased.
Type of cells involved	Somatic cells	Germline cells

Key concept

Cell division allows for the replication and transfer of genetic information in prokaryotes and eukaryotes. Prokaryotes divide by binary fission. Somatic cells of eukaryotes divide by mitosis. Germline cells of eukaryotes divide by meiosis.

Question set 2.2d

REMEMBERING

1 Name the eight main phases of meiosis.
2 Describe the role of cytokinesis I and II in meiosis.
3 What is the process of synapsis?

UNDERSTANDING

4 Draw a labelled diagram to show your understanding of crossing over.
5 State the phase in which independent assortment occurs and identify why it is important.
6 Distinguish between the daughter cells of mitosis and the daughter cells of meiosis.

APPLYING

7 Complete the following table for two different organisms in mitosis and meiosis.

FACTOR	HUMAN	TASMANIAN DEVIL
2*n* (diploid number)		14
Number of chromosomes in a parent cell	46	
Number of chromosomes in a somatic cell at the end of mitosis		
Number of chromosomes in a sex cell (gamete) at the end of meiosis		
Number of chromosomes at the end of telophase I		7

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2.3 FERTILISATION

Meiosis begins the process of transferring genetic information to the next generation by replicating DNA and producing male and female gametes. Fertilisation is the joining of the two gametes to form a zygote. The zygote receives one of each of its pairs of chromosomes from each parent. Fertilisation completes the transfer of genetic information to the next generation.

In the process of fertilisation, male and female haploid sex cells fuse to produce a diploid zygote. Two gametes from different individuals (usually one male and one female) of the same species need to combine to produce a new individual of that species. This is called sexual reproduction. Organisms produced by sexual reproduction have a different combination of DNA from that of either parent.

The zygote formed is a cell with approximately twice the amount of DNA that each gamete had. Meiosis halves the amount of DNA, but fertilisation restores the amount of DNA to the required amount for a particular species. In humans, the gametes produced by meiosis contain 23 chromosomes. Fertilisation restores the number of chromosomes to 46 (23 + 23 = 46), the number of chromosomes in somatic cells. Different species have different numbers of chromosomes. For example, many species of eucalyptus have 22 chromosomes, potatoes have 48 and hermit crabs have 254.

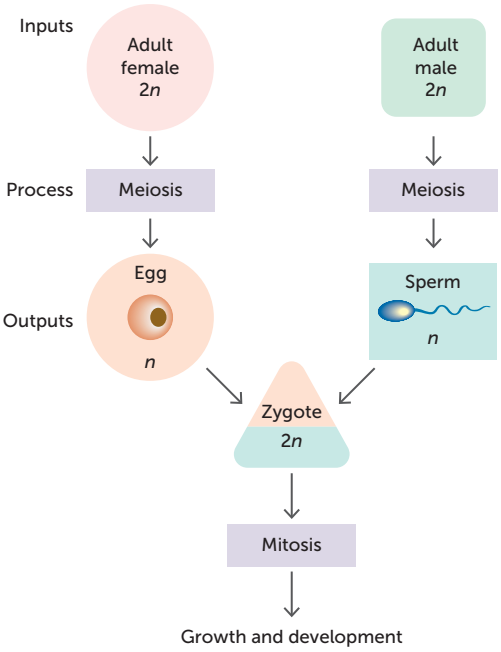

FIGURE 2.23 The inputs and outputs of meiosis

Key concept

Fertilisation leads to increased variation in offspring because it involves the fusing of two haploid cells from two different parents to form one diploid cell (the zygote).

Maths in biology

2.1 APPLICATION

During prophase I, maternal and paternal homologous chromosomes orient themselves randomly at the equator. Each maternal chromosome randomly positions itself closer to one pole than the other, and independently of the other maternal chromosomes. The number of possible orientations is equal to 2 raised to the power of the number of chromosome pairs. For example, for a haploid number of n, 2^n is the number of possible outcomes. Humans have a haploid number of 23, so the number of possible assortments is 2^{23}, which gives a value of over 8 million. This means that there are over 8 million possible combinations of chromosomes in a gamete from one individual, just from the random orientation of the homologous chromosomes. If we add the effects of crossing over, the number of combinations increases even further.

Question

Calculate the number of possible combinations of chromosomes in a Tasmanian devil's gametes ($n = 7$).

The role of the sex chromosomes

In humans, normally all female gametes contain 22 autosomes and an X chromosome. On the other hand, 50% of male gametes contain 22 autosomes and a Y chromosome and 50% contain 22 autosomes and an X chromosome. Thus, in humans there is a 50% chance that, in fertilisation, a

sperm cell bearing a Y chromosome will fuse with an egg cell, resulting in a male zygote (XY), and a 50% chance that a sperm cell bearing an X chromosome will fuse with an egg cell, resulting in a female zygote (XX).

Not all life continues

Cells do not live forever; they are pre-programmed to age and die after a given life span. For example, some skin cells known as keratinocytes live for about 3 weeks. The dead cells form a surface layer that is continually shed. Keratinocytes self-destruct in an orderly and programmed manner called **apoptosis**.

Far from being detrimental to an organism, cell death by apoptosis is a vital and formative process that is essential for development and the shaping of organs and tissues. Apoptosis can cause some cells to die at a particular stage of development. For example, dying cells enable a tadpole to lose its tail as it becomes a frog, and a human embryo to lose the webbing between its fingers and toes. In fact, almost all multicellular organisms have cells that are born to die.

Question set 2.3

REMEMBERING

1 Define
   - **a** fertilisation
   - **b** autosomes
   - **c** apoptosis.

2 What are the inputs and outputs of meiosis?

3 Describe the role of the sex chromosomes.

UNDERSTANDING

4 Explain why offspring produced from asexual reproduction resemble their parent, whereas offspring produced from sexual reproduction are different from their parents.

5 Is the process of apoptosis beneficial to an organism? Explain.

APPLYING

6 Explain why genetic material needs to halve when gametes are created, prior to fertilisation.

SCIENTIFIC LITERACY

Letting in sunlight can kill dark-loving bacteria, study shows

Prokaryotes multiply and survive under a range of conditions, but the conditions need to be appropriate for the particular type of prokaryote. Bacteria have been studied in light and dark conditions. In an article in the journal *Microbiome*, it was reported that an abundance of sunlight was significantly associated with lower amounts of certain types of bacteria. Researchers at the University of Oregon found that in dark rooms about 12% of bacteria, on average, were able to reproduce. In sunlight, only 6.8% thrived. That figure was down to 6.1% of bacteria exposed to UV light. Dr Ashkaan Fahimipour, the postdoctoral researcher in biology who conducted the study, commented during an interview with ABC News that this could actually have an impact on health. He and his colleagues found smaller communities of different types of bacteria grew under greater light exposure.

Questions

1 What were the independent and dependent variables in this study?

2 Write a conclusion for the study, using relevant data to support your answer.

3 Create a hypothesis for a factor other than light that may affect the rate of binary fission.

ABC News
Read the interview with Dr Fahimipour.

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CHAPTER 2 ACTIVITY

2.1 ACTIVITY

Staring mutations in the face: exploring the chromosomal anomalies of the devil facial tumour disease

As the world's largest living carnivorous marsupial, the Tasmanian devil (*Sarcophilus harrisii*) is an Australian icon. Sadly, since the mid-1990s a sizeable proportion of the wild population has succumbed to a rare and often fatal form of transmissible cancer called the devil facial tumour disease (DFTD) (Figure 2.24).

The cancer is spread between animals when they bite each other, often during courtship. Analysis of DFTD cells has demonstrated that tumour cells all have a similar karyotype (with just a few variations), even though they may have been isolated from genetically different animals from different locations, and that the DFTD karyotype is quite different from that of the Tasmanian devil host animals. Transmission relies on the recipient of the DFTD failing to mount an immune response against the foreign DFTD cells. This is now understood to be due to the tumour evolving a mechanism that prevents the Tasmanian devil's immune system from overcoming it.

Each strain of the cancer is thought to have arisen in a single host and then spread. The tumour cells themselves are the infectious agent being passed from devil to devil. A cytogeneticist at the Royal Hobart Hospital performed the karyotyping for several DFTD samples. Analysis of the samples revealed two different DFTDs, one originating from a female (DFTD1), and a more recent one originating from a male (DFTD2). Both DFTDs are transmissible, grow in a host via cloning, and have a different genetic makeup from the host cells. That research was reported in 2015, and since then at least two other strains have been found.

Mutations are described in further detail in Chapter 4

FIGURE 2.24 A Tasmanian devil infected with devil facial tumour disease

The Biology Corner 1
Construct a karyotype of Tasmanian devil chromosomes

The Biology Corner 2
Follow-up activity

Aim

To construct and compare two karyotypes for the Tasmanian devil – one for a healthy cell and the other for a cell taken from a DFTD tumour – and to consider what mutations may have occurred to give rise to DFTD. The cancer that causes DFTD is caused by an infectious pathogen. The fact that it can be transmitted is considered rare for cancer.

You will need

Pencil, scissors, glue, blank sheet of paper, photocopy of the chromosome images in Figure 2.25 (page 46), red and blue pens or pencils

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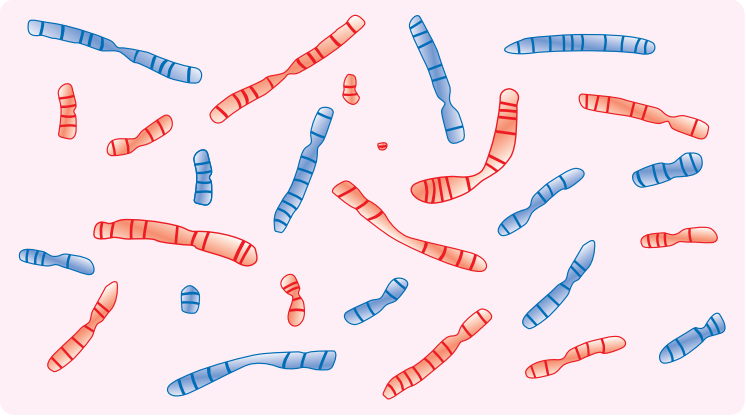

FIGURE 2.25 A mixture of the chromosomes from a healthy cell (red) and a DFTD cell (blue) from a Tasmanian devil

What to do

Referring to Figure 2.25, on a photocopy of the diagram, outline the chromosomes from the healthy cell in red, and those from the DFTD cell in blue.

1. Cut out each chromosome and add it to a red (healthy cell) pile or a blue (tumour cell) pile. Assemble the red cut-out chromosomes into a karyotype by pairing them and ordering them from largest to smallest.
2. Orientate a sheet of paper in landscape format. Secure the karyotype by gluing the chromosome pairs onto the top half of the paper, leaving a space of approximately 10 cm on the right-hand side. The largest chromosomes should be on the left, the smallest on the right.
3. Label the chromosome pairs by numbering the largest pair '1' and continue labelling them sequentially down to the second smallest pair. The two smallest chromosomes are the sex chromosomes, and should be labelled as such.

Now work on the 'blue' chromosomes from the DFTD cell.

4. Pair up any matching chromosomes. Leave aside any that cannot be paired.
5. Compare the paired chromosomes from the DFTD cell with those in the karyotype of the healthy cell. Arrange the paired chromosomes from the DFTD cell underneath the corresponding chromosomes from the healthy cell and glue them onto the paper.
6. Compare the unpaired chromosomes from the DFTD cell with those of the healthy cell. If any DFTD chromosomes look similar to any of those in the healthy karyotype, glue them onto the paper underneath the corresponding chromosomes.
7. You will be left with unpaired chromosomes from the DFTD cell that cannot be identified from the healthy karyotype. Arrange these from largest to smallest and glue them onto the page in the space to the right-hand side of the DFTD cell chromosomes.
8. Label the identifiable DFTD cell chromosomes, including any unpaired chromosomes, with the same labels as for the healthy cell. Label the remaining DFTD cell chromosomes on the right-hand side as 'M1', 'M2' etc. (M represents 'mutation'.)

What did you discover?

1. What features of the chromosomes did you use to match corresponding pairs?
2. What are the diploid and haploid numbers for healthy cells?
3. What is the sex of the animal from which the healthy cell was taken? How do you know?
4. What differences are there between the karyotypes of the healthy cell and the DFTD cell?
5. Discuss what changes might have occurred to the chromosomes of the Tasmanian devil to give rise to DFTD.

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CHAPTER 2 SUMMARY

Chapter 2 Activity sheet

- Cell division is essential for growth, development, repair and reproduction.
- Heredity is the study of the patterns and mechanisms of genetic inheritance through generations.
- Asexual reproduction results in limited genetic variation. Sexual reproduction combines genetic information from two parent cells and increases the genetic variation in offspring.
- In eukaryotic chromosomes, the DNA in the nucleus is coiled around the histone proteins to form nucleosomes.
- When matched and ordered, eukaryotic chromosomes can be displayed in a karyotype, and different chromosome sizes, centromere positions, and banding patterns can be observed.
- Somatic or body cells have pairs of homologous chromosomes; one chromosome of each pair comes from the male parent and the other from the female parent.
- Sex chromosomes that determine an individual's sex are generally matched in one sex (e.g. XX) and unmatched in the other sex (e.g. XY).
- A diploid number ($2n$) of chromosomes is found in somatic cells; a haploid number (n) (i.e. one of each pair of homologous chromosomes) is found in gametes.
- Homologous chromosomes have the same genes at the same position (locus), but there may be alternative forms of the gene, called alleles.
- Chromosomes in prokaryotic cells are generally circular and found in a region of the cell called the nucleoid.
- Small rings of DNA called plasmids may also be present in prokaryotic cells.
- Prokaryotic cells reproduce by binary fission.
- The sequence of events in cell division is called the cell cycle.
- Eukaryotic cell division involves nuclear division (mitosis or meiosis) and cytoplasmic division (cytokinesis).
- In mitosis, the cell divides once after passing through four phases (prophase, metaphase, anaphase and telophase). Cells formed by mitosis generally have the same genetic material as their parent cell.
- In meiosis, the cell divides twice (meiosis I and II). Cells formed by meiosis are called gametes.
- Fertilisation is the fusion of gametes from two different parent cells to form a new cell.

CHAPTER 2 GLOSSARY

Allele One of various versions of the same gene (at the same locus) distinguished by small differences in the DNA sequence

Apoptosis A programmed series of events that leads to cell death (as a result of the dismantling of the internal contents of the cell by various enzymes, including caspases)

Asexual reproduction The process by which a single parent produces offspring and that does not involve fusion of gametes; a process that usually results in identical offspring

Autosome A chromosome that is not a sex chromosome

Binary fission The division of a cell into two cells without mitosis; a prokaryotic cell undergoes binary fission to form two identical daughter cells; a form of asexual reproduction

Bivalent A structure (visible in a cell during prophase I of meiosis) made up of two homologous chromosomes joined together

Cell cycle An ordered sequence of events in the life of a cell from when it was formed from a parent cell until its own division

Cell division The splitting of a cell into two new functioning cells

Cell plate The structure produced by dividing plant cells in the place where the new cell wall is forming

Centriole A minute rod-shaped organelle present in many resting cells, just outside the

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nuclear membrane that helps make the spindle fibres for cell division; a centrosome contains two centrioles; it is usually absent in plants

Centromere The waist-like constriction in a chromosome where the spindle fibres attach; it enables the movement of chromosomes during cell division

Centrosome An organelle containing a pair of centrioles; it duplicates during cell division, while the DNA is duplicating, and the two centrosomes then separate to opposite poles of the dividing cell; it produces the spindle during cell mitosis and meiosis, and one of the centrosomes goes into each daughter cell

Chromatid Daughter strand of a duplicated chromosome that is joined to another chromatid by a centromere

Chromatin An organised, loosely coiled complex of DNA and its proteins that is found in eukaryotic non-dividing cells; it is more compact than the DNA of prokaryotes; chromatin supercoils to become the chromosomes observable during eukaryotic cell division

Chromosome A structure composed of DNA and protein that contains linear arrays of genes carrying genetic information; prokaryotes generally have one circular chromosome, whereas eukaryotes have a number of linear chromosomes

Cleavage The division of the cytoplasm in an animal cell

Cleavage furrow A shallow, ring-like depression that forms on the surface of an animal cell undergoing cytokinesis as contractile microfilaments pull the cell membrane inward; it defines where the cytoplasm will be divided to make two cells

Crossing over The exchange of genetic material between maternal and paternal homologous chromosomes (of non-sister chromatids) that occurs during the first step of meoisis (prophase I)

Cytokinesis The division of the cytoplasm immediately after mitosis, meiosis I or meiosis II to create two separate daughter cells

Differentiation The process during development whereby newly formed cells become more specialised as they mature; an example of cell differentiation is the development of root tip cells of plants into phloem, xylem and root hairs; during the process of differentiation, cells gain specialised structures and functions

Diploid (2*n*) Describes a cell or organism that has a genome that contains two copies of each chromosome; the diploid number of chromosomes is represented by $2n$

DNA (deoxyribonucleic acid) The information-containing molecule present in all living things that contains the instructions, written in a chemical code, for the production of proteins by the cell; the information it contains is sufficient for the making and maintaining of the organism; in addition, DNA is the genetic material that passes this information on to the next generation

Eukaryotic cell A complex cell containing membrane-bound organelles, including a nucleus

Fertilisation The fusion of haploid male and female gametes during sexual reproduction to produce a diploid zygote; the random union of gametes is known as random fertilisation

Gamete A male or female reproductive cell; one of each type combine at fertilisation; in humans, the gametes are ova and sperm cells; in flowering plants, pollen grains contain male gametes and ovules contain female gametes

Gene A unit of heredity that transmits information from one generation to the next; a segment of DNA that codes for a polypeptide

Genetic Refers to the mechanisms and patterns of inheritance; relating to the transmission of coded chemical instructions from one generation to the next

Genome All of the genetic material contained in an organism or a cell; it includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

Germline cell A specialised sex cell that gives rise to gametes; early in an embyro's development, its germline cells specialise into male or female germ cells

Haploid (*n*) Describes a cell or organism that has a genome that contains one copy of

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each chromosome; the haploid number of chromosomes is represented by *n*

Heredity The study of inheritance; the genetic transmission of characteristics from one generation to another

Heterosome One of the non-identical chromosomes that pairs up at meiosis (e.g. the X and Y chromosomes in male humans)

Histone A protein around which DNA winds in eukaryotic cells to form a nucleosome

Homologous chromosomes A pair of chromosomes of the same size and shape and that has the same genes at the same locations

Interphase The stage between nuclear divisions that involves metabolic activity, growth, and duplication of chromosomes

Karyotype A display of the number and appearance of the chromosomes of an organism or cell as observed at metaphase

Locus (plural loci) The position a gene occupies on a chromosome

Maternal chromosome The chromosome in a pair of chromosomes that came from the mother

Meiosis A type of cellular division in sexually reproducing organisms that involves two rounds of cell division, but only one round of DNA replication; during meiosis, the chromosome number of a cell is halved so that the daughter cells are haploid; meiosis is the basis of gamete formation in some plants and animals and of spore formation in other plants

Mitosis A type of nuclear division in somatic cells that maintains the parental diploid number of chromosomes in the daughter cells; it is the basis of bodily growth and of asexual reproduction in many eukaryotic species

Nitrogenous base A structural component of the nucleotides that make up DNA or RNA

Nucleoid The region within a prokaryotic cell that contains the genetic material

Nucleolus A structure found within the nucleus of a non-dividing cell; a site in which ribosomes are made from protein and RNA subunits

Nucleotide The basic building block of nucleic acids (DNA and RNA); nucleotides are linked together by phosphodiester bonds; each nucleotide is made up of a five-carbon sugar, a phosphate group and a nitrogenous base

Nucleus The dense organelle of a eukaryotic cell that contains genetic material in the form of chromosomes and is enclosed by a nuclear membrane; in its resting phase, the genetic material takes the form of loosely coiled chromatin; the chromatin supercoils and condenses to form chromosomes before cell division

Organelle A specialised part of a cell that has its own specific function; a 'little organ'

Paternal chromosome The chromosome in a pair of chromosomes that came from the father

Plasmid A small circular piece of DNA, found in bacteria, that is able to replicate independently of the cell's chromosomes; engineered plasmids may carry antibiotic-resistance markers

Prokaryote A single-celled organism that lacks membrane-bound organelles such as a nucleus

Sex chromosome A chromosome that determines the sex of an organism and affects sexual traits

Sexual reproduction A form of reproduction in which offspring are produced from two parents by the fusion of male and female gametes

Somatic cell A body cell that is not a germ cell

Synapsis The pairing of homologous chromosomes during prophase I of meiosis

Trait An inheritable characteristic; phenotype

Zygote The first cell of a new individual; it is formed by fusion of a male and a female gamete (fertilisation) during sexual reproduction

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CHAPTER 2 REVIEW QUESTIONS

Remembering

1 Draw and label a chromosome. Include the following labels: locus, chromatid, centromere, chromosome.
2 Explain how a karyotype can indicate the sex of an organism.
3 Describe the movement of genetic material in a cell during the cell cycle.

Understanding

4 The amount of nuclear DNA in any given cell can be measured quite accurately. Predict the stages of the cell cycle in which you would expect to see changes in the amount of nuclear DNA.
5 Compare and contrast binary fission with mitosis.
6 Compare and contrast the concept of a chromosome with the concept of a gene.

Applying

7 Predict what would happen if cytokinesis did not occur during a cell cycle.

Analysing

8 When animals of different species are kept together in captivity, they sometimes mate and produce offspring. A donkey has a diploid number of 62, and a zebra has a diploid number of 44.
   a Name the type of cell in the donkey that would be expected to contain 31 chromosomes.
   b Name the type of cell in the zebra that would be expected to contain 44 chromosomes.
   c Estimate how many chromosomes you would expect to find in the somatic cells of the donkey.
   d If a 'zonkey' (a hybrid formed by the fertilisation of a female donkey egg by a zebra sperm) is produced, predict its $2n$ number.
   e Describe how a zonkey karyotype would differ from the karyotype of a zebra.
   f Suggest problems that might occur when the zonkey produces gametes.
   g Explain why most hybrid animals are infertile.
9 Draw a simple table that compares mitosis and meiosis. Include the number of daughter cells produced by each parent cell, the number of chromosomes in each daughter cell, and whether the daughter cells are identical or non-identical.

Evaluating

10 All chromosomes are double stranded and linear in shape. Do you agree with this statement? Justify your answer.
11 'Cells produced by meiosis only contain half the amount of DNA compared with their parent cells. This means DNA does not replicate during meiosis.' Do you agree with this statement? Justify your answer.
12 Explain why meiosis is necessary for gamete formation, rather than mitosis.
13 A group of cells being studied were never observed to undergo division. Explain whether this means the cells were dead. Justify your answer.
14 Prokaryotes divide by means of binary fission and generally produce cells identical to one another and to the parent cell. Complex organisms produce sex cells that combine to form a new individual. Identify an advantage and a disadvantage for each of asexual and sexual reproduction.

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Creating and reflecting

15 Reflect on what you have learned about mitosis and meiosis. Draw a mind map to summarise your knowledge.

PRACTICE EXAM QUESTIONS

1 Bacterial cells reproduce by:
- **A** binary fission only
- **B** meiosis only
- **C** binary fission and mitosis
- **D** mitosis and meiosis.

[Q4. 2017 SCSA]

2 Crossing over is the:
- **A** exchange of alleles between homologous chromosomes
- **B** exchange of alleles between non-homologous chromosomes
- **C** segregation of homologous chromosomes to different poles
- **D** segregation of non-homologous chromosomes to different poles.

[Q27. 2017 SCSA]

3 In mitosis, a parent cell usually produces:
- **A** four daughter cells, each of which has the same number of chromosomes as the parent cell
- **B** four daughter cells, each of which has half the number of chromosomes as the parent cell
- **C** two daughter cells, each of which has the same number of chromosomes as the parent cell
- **D** two daughter cells, each of which has half the number of chromosomes as the parent cell.

[Q11. 2016 SCSA]

4 Meiosis produces:
- **A** four diploid gametes
- **B** two haploid gametes
- **C** two diploid gametes
- **D** four haploid gametes.

[Q6. 2014 SCSA]

Use the following information to answer questions 5 and 6.

The diploid numbers of chromosomes in the horse and donkey are 64 and 62, respectively. A mule is the offspring of a cross between a horse and a donkey. Mules survive but are sterile because they cannot produce functional gametes.

5 On the basis of the above information, how many chromosomes would be present in a diploid cell of a mule? Explain your answer. (4 marks)

[Q32a. 2018 SCSA]

6 Explain why mules cannot produce functional gametes. (4 marks)

[Q32b. 2018 SCSA]

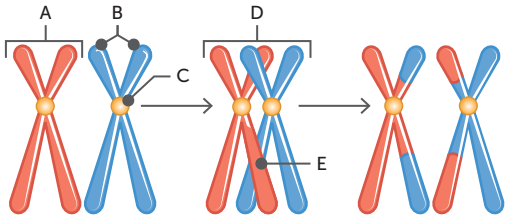
7 Name and describe the process by which a bacterial cell reproduces. (4 marks)

[Q31a. 2016 SCSA]

8 Describe the process of meiosis and explain how this process produces genetic variation. (10 marks)

[Q36b. 2016 SCSA]

Look at the diagram below and answer the questions that follow.



9 Provide labels A to D for the diagram. (4 marks)

[Q31a. 2014 SCSA]

10 Name the process occurring at E and explain the biological importance of the process. (3 marks)

[Q31b. and c. 2014 SCSA]

3

DNA STRUCTURE AND FUNCTION

CHAPTER 3 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Describe the structure of DNA, including the following:
   - **a** shape
   - **b** names of the matching bases
   - **c** three main components that make up the molecule. Hint: two form the 'backbone' of the molecule.
2 How does the unique structure of DNA enable it to perform its functions?
3 List three roles of DNA in a cell.

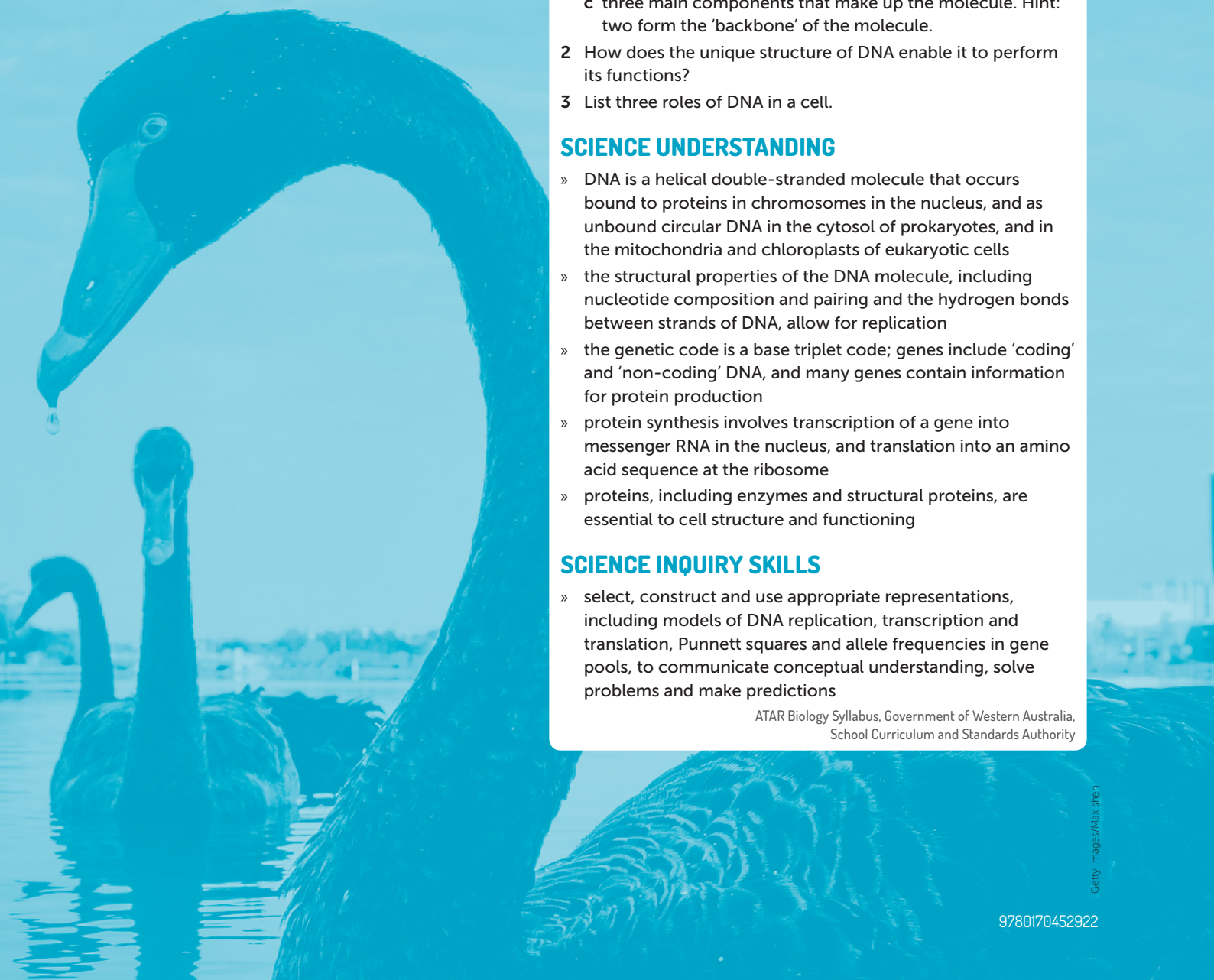
SCIENCE UNDERSTANDING

- DNA is a helical double-stranded molecule that occurs bound to proteins in chromosomes in the nucleus, and as unbound circular DNA in the cytosol of prokaryotes, and in the mitochondria and chloroplasts of eukaryotic cells
- the structural properties of the DNA molecule, including nucleotide composition and pairing and the hydrogen bonds between strands of DNA, allow for replication
- the genetic code is a base triplet code; genes include 'coding' and 'non-coding' DNA, and many genes contain information for protein production
- protein synthesis involves transcription of a gene into messenger RNA in the nucleus, and translation into an amino acid sequence at the ribosome
- proteins, including enzymes and structural proteins, are essential to cell structure and functioning

SCIENCE INQUIRY SKILLS

- select, construct and use appropriate representations, including models of DNA replication, transcription and translation, Punnett squares and allele frequencies in gene pools, to communicate conceptual understanding, solve problems and make predictions

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority



Getty Images/Max shen

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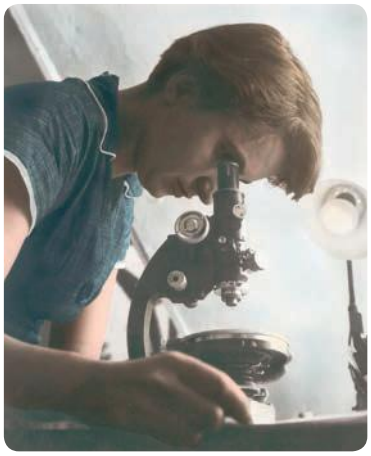
3.1 THE DISCOVERY OF DNA

Many secrets of **DNA (deoxyribonucleic acid)** were unlocked in 1952 at King's College, when Rosalind Franklin took the first clear X-ray diffraction image of DNA. Franklin's photograph helped confirm the spiral nature of DNA. Without her consent, her colleague Maurice Wilkins took her photographs to James Watson and Francis Crick. The photographs gave them evidence for the 3D structure they had previously theorised for DNA. Using these results and other accumulated evidence, Watson and Crick suggested that DNA consists of the now familiar two strands, resembling the uprights of a ladder, linked by 'rungs' (made of the four types of **nucleotides**), twisted to form a **double helix**. Rosalind Franklin had already died when Wilkins, Watson and Crick received a Nobel prize for their work in discovering the structure of DNA.

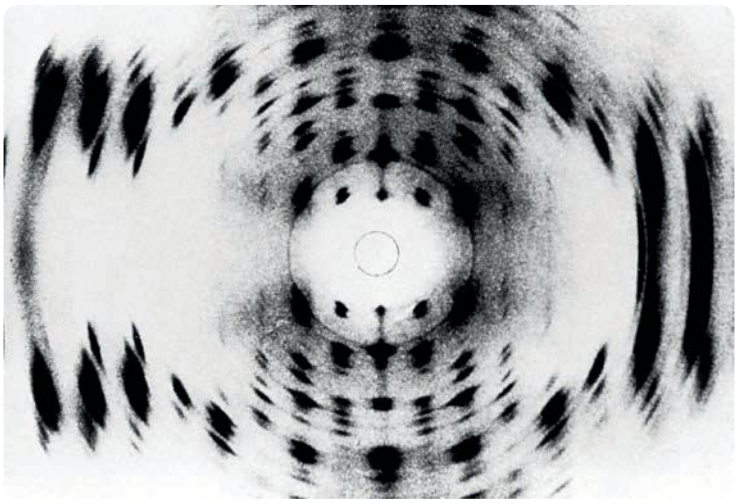


Alamy Stock Photo/Science History Images

FIGURE 3.1 Rosalind Franklin, an expert X-ray crystallographer, worked on the structure of DNA in the early 1950s. Her work was pivotal in enabling Watson and Crick to propose their hypothesis for the structure of DNA.

It is now known that nucleic acids such as DNA do form a double helix: two linear strands, each containing a sequence of nucleotide subunits, twisted together into a spiral.

Some years earlier, an Austro-Hungarian biochemist working in the USA, Erwin Chargaff, used a technique called chromatography to work out the ratios of the four types of **nitrogenous bases** [adenine (A), cytosine (C), guanine (G) and thymine (T)] present in the nucleotide subunits. He found that the amount of guanine was equal to the amount of cytosine, and the amount of adenine was equal to the amount of thymine.



Alamy Stock Photo/Science History Images

FIGURE 3.2 An X-ray diffraction photograph of DNA. The DNA molecule was too small to see using conventional methods, so X-rays were used. The image produced an accurate 3D shape.

The pairs of nitrogenous bases are known as complementary base pairs. Complementary pairing is the phenomenon whereby guanine always hydrogen bonds with cytosine, and adenine always hydrogen bonds with thymine. Guanine and cytosine share three hydrogen bonds, and adenine and thymine share two hydrogen bonds. The complementary pairing helps produce the 3D helical structure of DNA. Nucleotides are the base units of DNA.

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Where is DNA found in eukaryotes and prokaryotes?

DNA occurs bound to **proteins** in **chromosomes** within the nucleus of eukaryotic cells. The nucleus is enclosed in a nuclear membrane to protect its interior. DNA is also found in prokaryotes, but as unbound circular DNA in the **nucleoid** region of the cytosol. The nucleoid region is not bound by a nuclear membrane, and therefore the DNA is not contained like it is in a eukaryotic cell. Unbound, circular DNA is also found in the mitochondria and chloroplasts of eukaryotic cells.

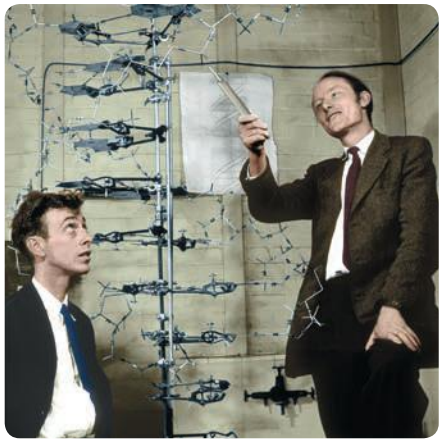

FIGURE 3.3 Watson (left) and Crick in 1953 with their model of part of a DNA molecule

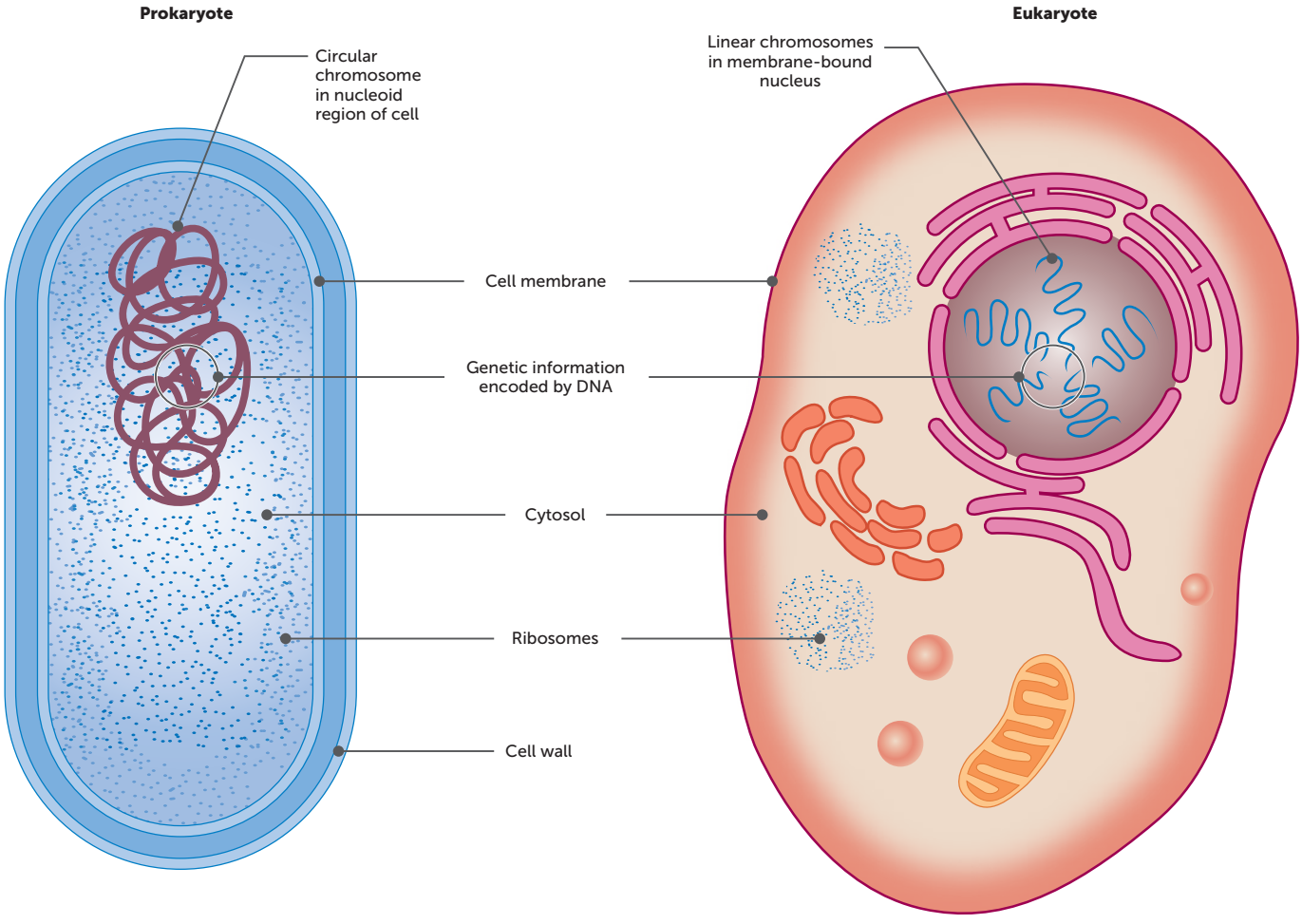

FIGURE 3.4 Structural similarities and differences between a prokaryotic cell and a eukaryotic cell

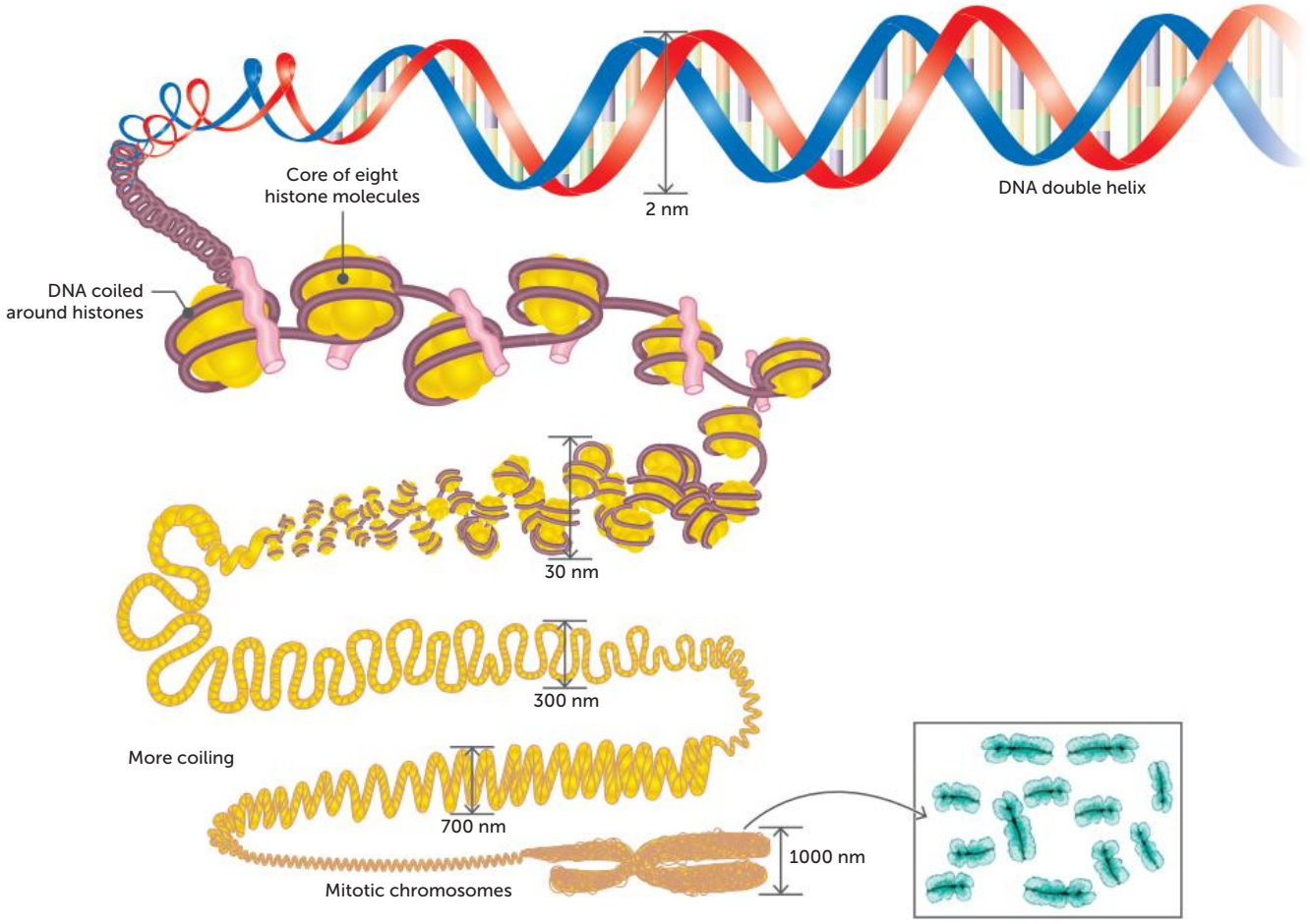

FIGURE 3.5 In the nucleus of eukaryotes, linear DNA is found bound to proteins and becomes tightly coiled to form chromosomes.

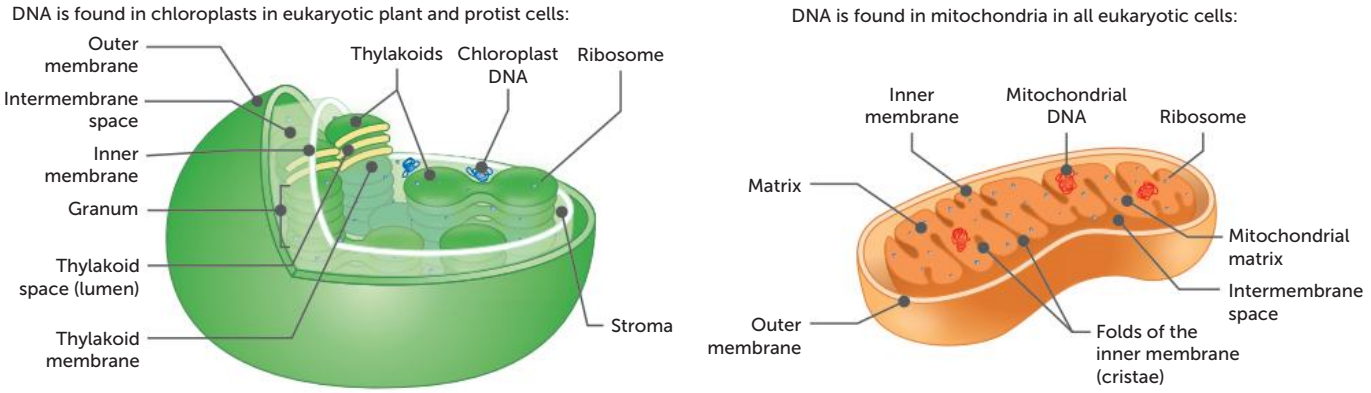

FIGURE 3.6 Circular DNA, which is not bound to proteins, is found in chloroplasts and mitochondria.

Key concept

DNA is a double-stranded helical molecule. In eukaryotic cells, DNA is found in a linear form bound to proteins in the nucleus, and in an unbound circular form in chloroplasts and mitochondria. In prokaryotic cells, DNA is found in an unbound circular form in the nucleoid region of the cytosol.

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3.1

APPLICATION

DNA and who it gets passed to

DNA contains instructions that make each species unique, and it is passed from parent cells to daughter cells during cell division, and from adults to their offspring during reproduction. In sexual reproduction, organisms inherit half of their nuclear DNA from the male parent and half from the female parent. However, organisms inherit all of their mitochondrial DNA from the female parent. This occurs because only egg cells, and not sperm cells, keep their mitochondria during fertilisation.

Question set 3.1

REMEMBERING

1 List the scientists mentioned so far in this chapter who contributed to the discovery of the structure of DNA.

2 Describe Watson and Crick's model of DNA.

UNDERSTANDING

3 Explain why DNA is not found in the nucleus of a prokaryotic cell.

4 Copy and complete the table of complementary base pairs in DNA.

NITROGENOUS BASE	COMPLEMENTARY BASE PAIR
A	
C	
T	

ANALYSING

5 Copy the following table and complete it using Chargaff's ratio of nucleotide subunits.

ADENINE	CYTOSINE	THYMINE	GUANINE
7	3	7	
21	25		25
		43	44

6 The average percentage composition of adenine in human DNA is 30%. Predict the percentage of the other three nucleotides.

3.2 STRUCTURAL PROPERTIES OF THE DNA MOLECULE

Nucleotides – the building blocks of DNA

DNA is a nucleic acid made up of nucleotides. Each nucleotide consists of three parts: a five-carbon (pentose) sugar known as deoxyribose sugar, a phosphate group and a nitrogenous base (adenine, cytosine, guanine or thymine). A nucleotide is the basic structural unit of DNA.

Each phosphate group is attached to two sugar molecules by 'ester' bonds and is then called a **phosphodiester bond** (see Figure 3.8). The five carbon atoms in each sugar molecule,

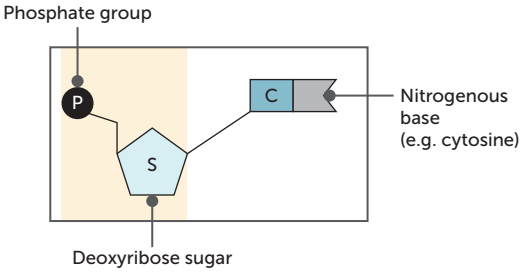

FIGURE 3.7 A nucleotide

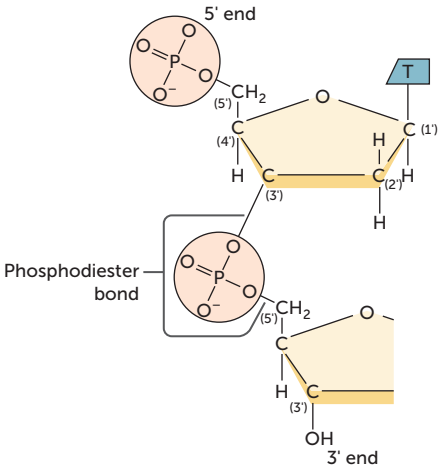

FIGURE 3.8 A phosphodiester bond

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which form a ring, are numbered 1' to 5'. One of the ester bonds is formed with the 3' carbon of one sugar ring and the other is formed with the 5' carbon of the next sugar ring. The chain of alternating sugar molecules and phosphate groups is called the sugar–phosphate backbone.

RNA (ribonucleic acid) has a similar structure to DNA, except deoxyribose sugar is replaced with ribose sugar.

A strand of nucleotides has directionality described using the phrase **5' to 3'**. The 5' end starts with a phosphate and the 3' end finishes with a sugar. DNA and RNA synthesis occurs in the 5' to 3' direction.

The structure of the DNA molecule

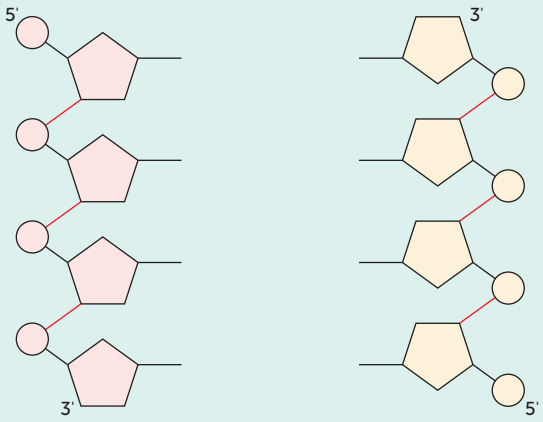
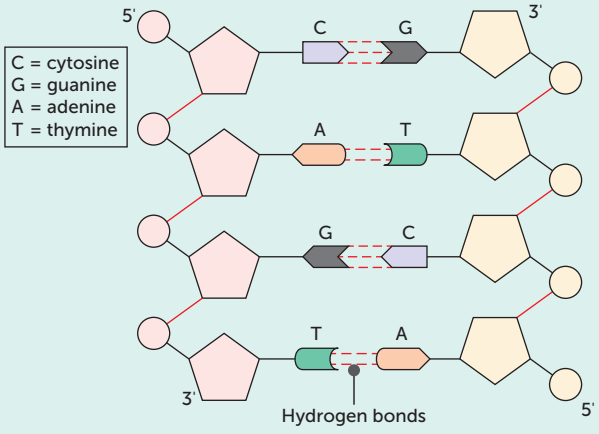
The shape of a DNA molecule is a double helix. The term 'double' refers to the two strands, which are joined by the weak hydrogen bonds between complementary pairs of nitrogenous bases. The **complementary base pairing** means that adenine always pairs with thymine, and cytosine always pairs with guanine. The term 'helix' describes the helical (spiral) molecular shape: the two linear strands run in opposite directions to each other (i.e. are anti-parallel) and are twisted into a helix.

Build DNA
Practise building DNA using complementary pairing.

Drawing and labelling DNA

Drawing and labelling DNA structure is an essential component of the course. This takes practice. Follow the steps below as you draw your own DNA structure. If you can draw it, then you know it!

TABLE 3.1 Steps for how to draw and label DNA structure

STEP	DRAW AND LABEL
1 Draw the anti-parallel deoxyribose sugars (pentagons) for the two strands. On one strand draw them upright, and on the other strand draw them upside down to show they are anti-parallel. Then draw the phosphodiester bonds (circles, each with a 'red' and a 'black' bond) connecting the sugars in a chain. The 'red' covalent bond joins each phosphate to a 3' carbon, and the 'black' covalent bond joins it to a 5' carbon.	 **FIGURE 3.9** Two anti-parallel strands of DNA
2 Draw the complementary base pairs. Indicate the weak hydrogen bonds with dotted lines: 2 hydrogen bonds between adenine and thymine and 3 hydrogen bonds between cytosine and guanine.	 **FIGURE 3.10** Complementary bases are paired
3 Make a key listing the full names of the nitrogenous bases and their complementary pairs.	

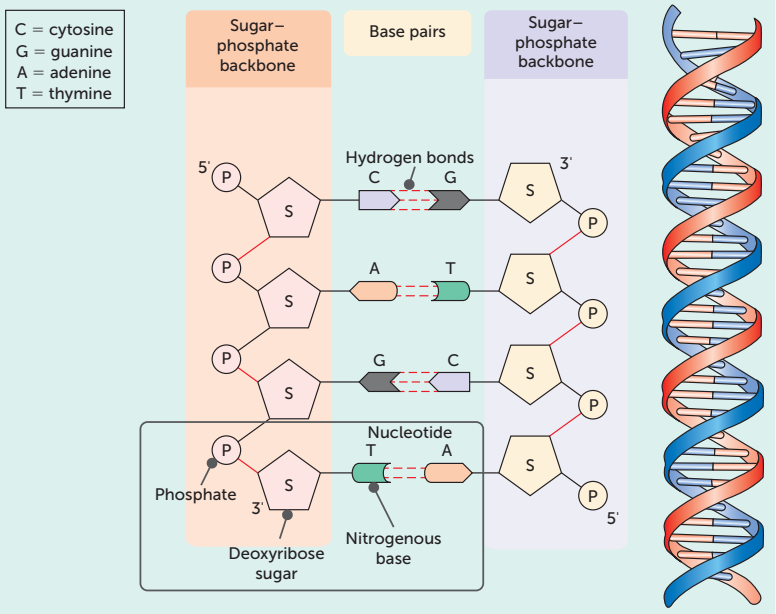
STEP	DRAW AND LABEL
4 Circle one nucleotide. Label its three parts. Nucleotides are very important because they are the building blocks of each strand.	
5 Label the sugar–phosphate backbone sections of the molecule, base pairs, 'phosphate', 'nucleotide' and 'deoxyribose'. In each sugar symbol write an 'S', and in each phosphate circle write a 'P'.	
6 Next to the chemical structure you have just drawn, draw a simplified smaller-scale DNA molecule, demonstrating its double-helix structure.	

FIGURE 3.11 The sugar–phosphate backbones and the double-helix structure

Key concept

The base unit of DNA is a nucleotide, which consists of one nitrogenous base, one deoxyribose sugar and one phosphate group. The two strands of DNA are held together by weak hydrogen bonding between the complementary nitrogenous bases: adenine and thymine, cytosine and guanine.

The structure of DNA enables it to function

DNA is the genetic material common to all organisms. It carries information coded in the segments of its molecule known as **genes**. DNA thus enables certain **traits** to be passed on to the next generation. A trait is an inheritable characteristic.

DNA is chemically the same in all organisms, although different species usually have different proportions of the various nucleotides, and each organism has a unique DNA sequence. The DNA sequences of individuals within a species have a lot more similarity than those of individuals of different species. In addition, DNA molecules in eukaryotes and prokaryotes generally differ in their associated proteins and in overall shape and appearance.

We now understand how DNA is transmitted between generations, how genes are controlled and how differences in genes can cause changes in the way organisms develop and behave. This knowledge has allowed us to manipulate genes to achieve desired characteristics. New technologies have enabled us to accurately examine the interrelationships between species and to account for changes that have occurred in species over time.

DNA stores the code for making proteins, and the inheritance of particular gene variants causes an individual to have a specific combination of proteins in its makeup. A section of DNA that codes for a specific protein (or **polypeptide**) is called a gene. It is now known that genes may code for more than one kind of polypeptide, and that genes interact with one another, causing changes in their expression (i.e. in the production of proteins). DNA, therefore, controls the growth and development of an organism.

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The structural properties of the DNA molecule [its nucleotide composition, pairing and hydrogen bonding (see Figure 3.12b)] are what allow **DNA replication** to occur. This is because the DNA strands can function as **template** strands.

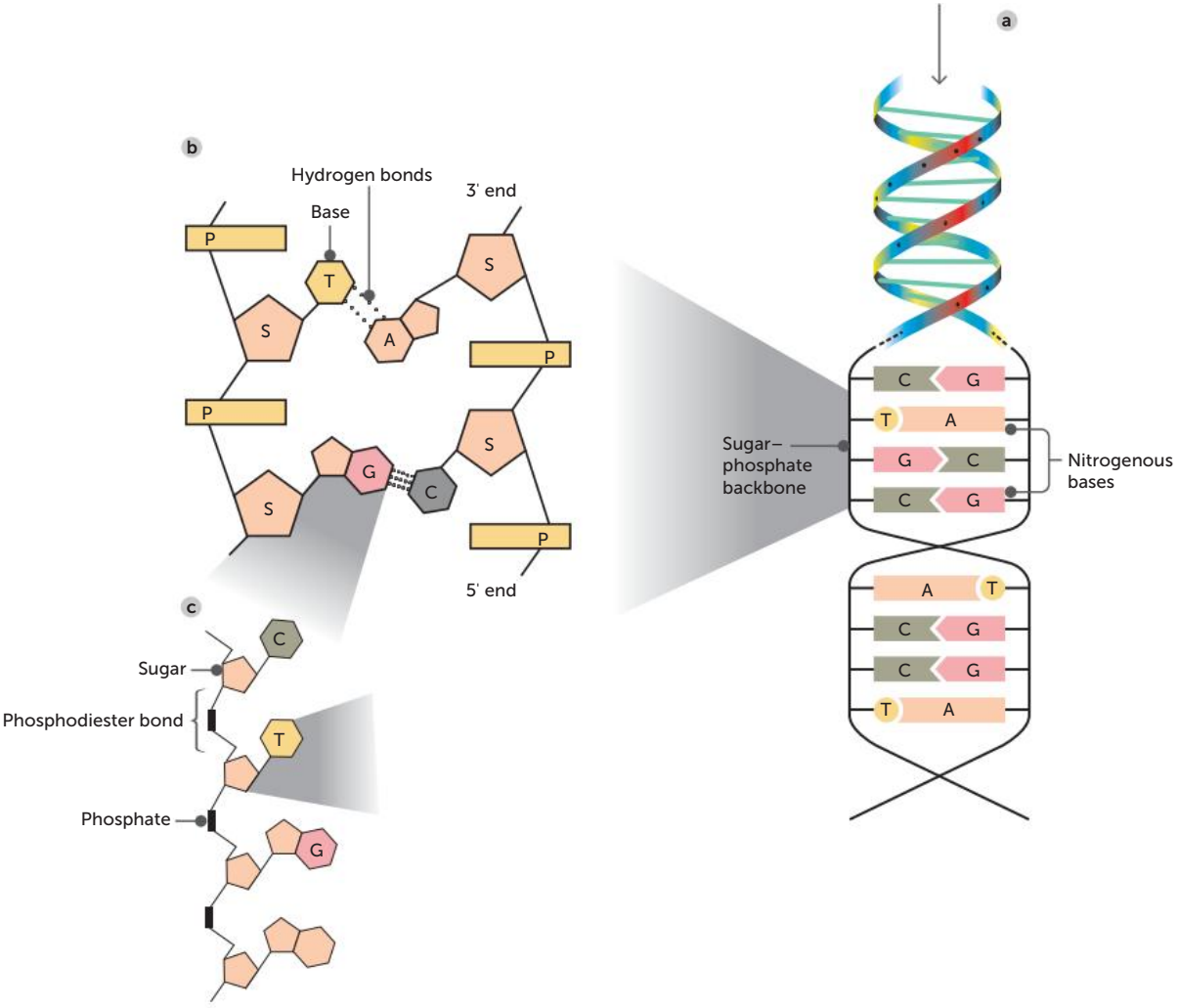

FIGURE 3.12 a The DNA helix is a double-stranded molecule. **b** The two strands are held together by hydrogen bonding between complementary nitrogenous bases. **c** As well as nitrogenous bases, nucleotides have a sugar–phosphate backbone, in which the sugar molecules are linked by phosphodiester bonds.

Question set 3.2

REMEMBERING

1 State the three components of a DNA nucleotide.

2 Draw and label your own diagram of DNA, following the instructions found in Table 3.1 (page 57).

3 What is one main difference between the structure of DNA and RNA?

UNDERSTANDING

4 Explain what is meant by the term 'anti-parallel'.

ANALYSING

5 Relate DNA's structure to three of its functions.

DNA structure
View this link to reinforce your learning.

DNA structure and replication

DNA learning centre

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CASE STUDY

DNA: further accumulation of knowledge

When the structure of DNA was deduced, it seemed to be the final piece of a biological puzzle. It taught us how hereditary information (the passing on of which was demonstrated by Mendel) was encoded. Between the 1950s and 1980s, scientists studying DNA thought most of our DNA was useless. They knew that small sections of DNA, known as genes, seemed to code for proteins, but they wondered about the other sections of the DNA. We now know genes can interact with one another and with the environment to result in traits, but scientists still do not know what most of an organism's DNA does.

In the 1950s–1960s, Watson and Crick and other collaborators had determined that DNA guides the production of RNA, and RNA guides the production of protein, which may then be manifested as an observable characteristic. But the biology of DNA is much more complex than was initially thought.

Researchers working for the Encyclopedia of DNA Elements (ENCODE) project, a public research consortium launched by the US National Human **Genome** Research Institute, have been mapping the parts of human chromosomes that are transcribed (copied). In addition, they have been studying how the copying of DNA is regulated and how the process is affected by the way the DNA is packaged. In 2012, they found that it is not only the roughly 1% of our DNA that contains genes that code for proteins that has a function. The **non-coding DNA** (DNA that does not code for protein) appears to have very important roles, such as regulatory and structural functions. ENCODE scientists are theorising that 80% of 'junk DNA' is active. See Coding and non-coding DNA, page 63.

Today, scientists analyse DNA for many purposes other than the study of **heredity**. **Genomics**, such as is researched at the Australian Museum's Australian Centre for Wildlife Genomics, is the study of the entire DNA sequence of an organism and of its genes. Scientists know how to sequence the DNA (work out the order of nucleotides). They do this for a range of purposes, such as identification of individuals, paternity testing, sex determination, measure of species relatedness, conservation, population management and forensics.

Questions

1. Define the term 'knowledge accumulation' and apply it to DNA discoveries.
2. List five uses of DNA analysis.
3. Explain how Franklin's first clear X-ray diffraction image of DNA laid some of the groundwork for the current uses of DNA analysis. (Hint: structure can indicate the mechanisms for function.)

3.3 DNA STRUCTURE ENABLES DNA REPLICATION

DNA contains the **genetic code** that determines the structure and function of all living things. The product of DNA replication is two identical, double-helix DNA molecules, each consisting of one parental strand and one new strand. DNA replication is referred to as **semi-conservative replication** because one of the two strands is conserved, or retained, from one generation to the next, while the other strand is new. DNA replication occurs during the S phase of interphase during the cell cycle (see 'The cell cycle' in Chapter 2, pages 31–32).

The purpose of DNA replication is to duplicate the code it carries. The code can then be passed on to daughter cells. In eukaryotic cells, the chromosomes gain a sister chromatid and become double stranded. DNA replication occurs in preparation for mitosis and meiosis.

Key concept

DNA replication is semi-conservative. Each of the new DNA molecules that are produced have one strand that is conserved (i.e. the parental strand) and one that is new (i.e. the daughter strand).

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DNA replication begins with an **enzyme** called **DNA helicase** 'unzipping' the long molecule of double-stranded DNA by breaking the weak hydrogen bonds between the nucleotides and thus exposing the nucleotide bases. This separation of the parental DNA strands happens along a small section at a time. The hydrogen bonds that hold the two strands of the DNA molecule together are weak, and the enzyme is easily able to separate them.

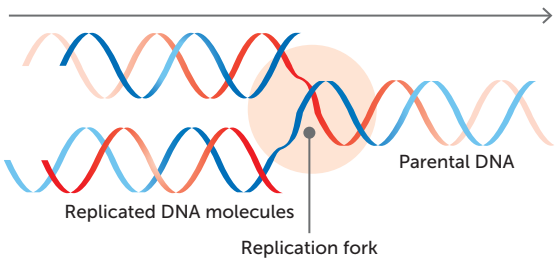

FIGURE 3.13 Movement of the replication fork along parental DNA causes unwinding of the original DNA double strands and rewinding of the two newly replicated double strands.

The junction between the unwound single strands of DNA and the intact double helix is called the **replication fork**. The replication fork moves along the parental DNA strand so that there is a continuous unwinding of the parental strands (Figure 3.13). Within the nucleus, stockpiles of free nucleotides attach to the exposed bases, according to the base-pairing rule (Figure 3.14), with the help of the enzyme **DNA polymerase**. Another enzyme, **DNA ligase**, seals the new short stretches of nucleotides into a continuous double strand that rewinds. Ligase catalyses the formation of phosphodiester bonds. The nucleotides link together in what is called a 5' to 3' direction, forming long molecules.

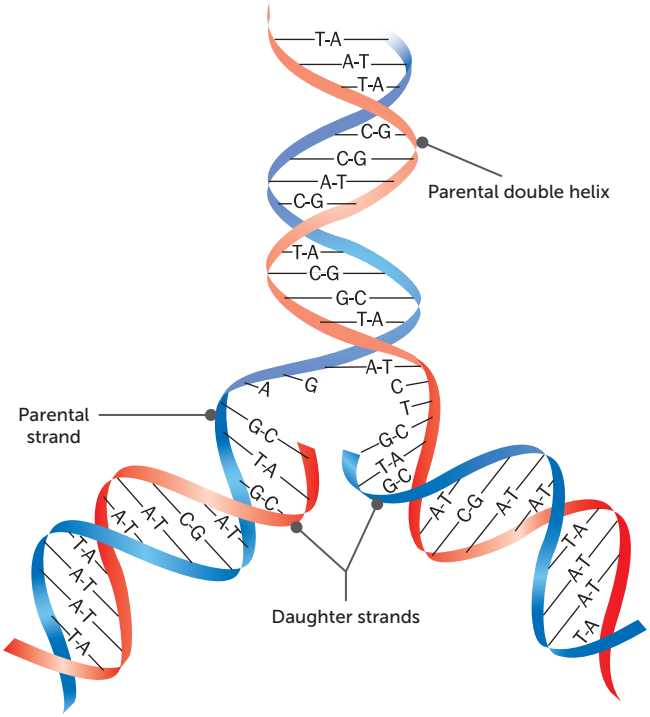

FIGURE 3.14 Replication of DNA. The specific relationships between A and T and between C and G ensure that the sequence of bases in the daughter DNA is exactly the same as that in the parent DNA.

As DNA strands are antiparallel, DNA polymerase moves in opposite directions on the two strands during synthesis. On the **leading strand**, DNA polymerase is moving towards the replication fork and synthesises continuously. On the **lagging strand**, DNA polymerase is moving away from the replication fork and synthesises in pieces called **Okazaki fragments**. The process of DNA replication is summarised in Table 3.2 (page 62).

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TABLE 3.2 DNA replication

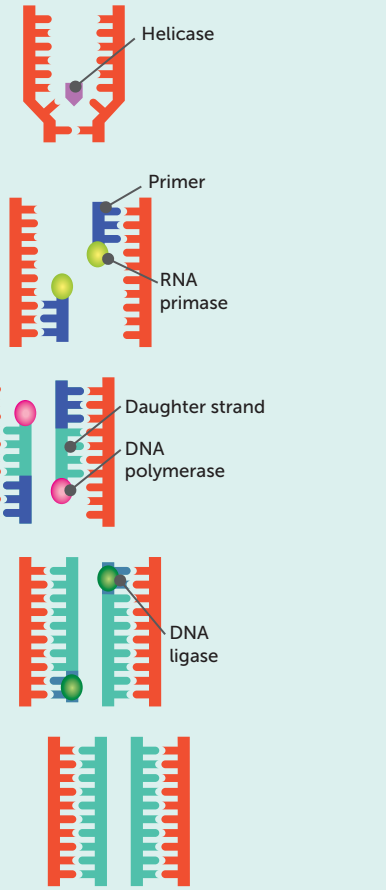
STEPS	VISUAL AID
1 DNA helicase unwinds and separates the double strand by breaking the weak hydrogen bonds between complementary base pairs. Each half of the parent molecule is used as a template.	
2 The enzyme RNA primase attaches a short sequence of RNA, known as a primer, to show DNA polymerase where to start adding nucleotides.	
3 Free nucleotides are added by DNA polymerase according to complementary base-pairing rules. Synthesis of the new daughter strand is in a 5' to 3' direction. Adenine pairs with thymine, and cytosine pairs with guanine.	
4 DNA ligase removes and replaces the primers. The result is two identical DNA molecules that are each made of one original parent strand and one new daughter strand. DNA replication is described as semi-conservative.	
5 In eukaryotic organisms, two identical sister chromatids are now ready for cell division. In prokaryotes, two identical circular chromosomes are now ready for binary fission.	

FIGURE 3.15 DNA replication involves enzymes

Figure 3.16 illustrates the continuous and discontinuous synthesis of DNA along each of the strands. Synthesis is continuous along the leading strand, with additional nucleotides being added one after the other. It is discontinuous along the lagging strand because it is a 3' to 5' strand and DNA polymerase can only synthesise new DNA in a 5' to 3' direction. Primers are attached at short intervals, starting from the replication fork. DNA polymerase synthesises short strands of new DNA starting at each primer, in a 5' to 3' direction. The short strands are called Okazaki fragments.

DNA polymerase moves in opposite directions on the two anti-parallel parent strands. DNA polymerase removes the RNA primers and replaces them with DNA nucleotides. DNA ligase joins the Okazaki fragments together to create a continuous strand. Ligase catalyses the formation of a phosphodiester bond.

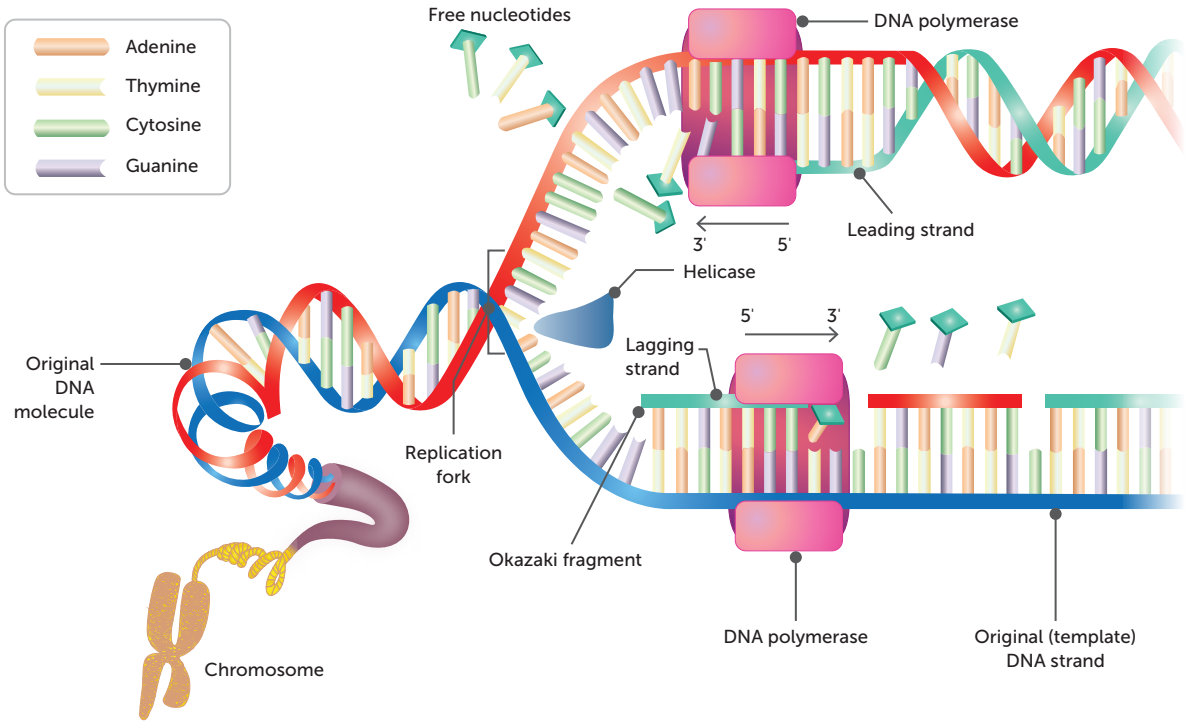


FIGURE 3.16 DNA replication showing the leading and lagging strands

Question set 3.3

REMEMBERING

1 State the role of DNA helicase, polymerase and ligase in DNA replication.

2 Draw a flow diagram to summarise the process of DNA replication. The one below has been included as an example of how to set it out.

UNDERSTANDING

3 Explain why the process of DNA replication is described as semi-conservative.

ANALYSING

4 Describe the relationship between DNA replication and cell division.

5 Predict the nucleotide sequence for the complementary strand of a fragment of a DNA chain with the nucleotide bases GCCTATTGCA.

DNA replication
View the link to reinforce your understanding of DNA replication.
DNA structure and replication

3.4 CODING AND NON-CODING DNA

DNA is a molecule consisting of a sequence of nucleotides. The entire order of the nucleotides in a human cell's DNA have been sequenced. The sequence of consecutive DNA 'letters' spanning all the chromosomes of a cell from start to finish is known as the **genome sequence**. Some sections of the sequence code for proteins and are called **coding DNA**. The coding DNA sections are also called genes. The coding DNA specifies sequences of **amino acids**, which are the building blocks of proteins. Proteins are responsible for nearly all cell functions. Humans have around 20 000 protein-coding genes. (Corn has around 32 000 genes and *Escherichia coli* (*E. coli*) bacteria has around 4400 genes.) Approximately 1–2% of the DNA in a human is comprised of coding DNA. Genes contain information

for the production of proteins, and proteins are the link between the stored genetic code, the genotype, and observable traits, called the phenotype.

The majority of the human genome is comprised of non-coding DNA. The German botanist Hans Winkler invented the term 'genome' in 1920 by combining the words GENe and chromosOME. A short definition of genome is 'all the DNA in a cell', and this includes the genes and also DNA that is not part of any gene. The sections of DNA that do not code for a protein are classified as non-coding DNA. Some non-coding DNA is transcribed into functional non-coding RNA molecules, such as transfer RNAs and regulatory RNAs. Historically, non-coding DNA was referred to as 'junk DNA', but through recent advances in knowledge, scientists have found that some of the non-coding DNA is important and therefore not actually 'junk'.

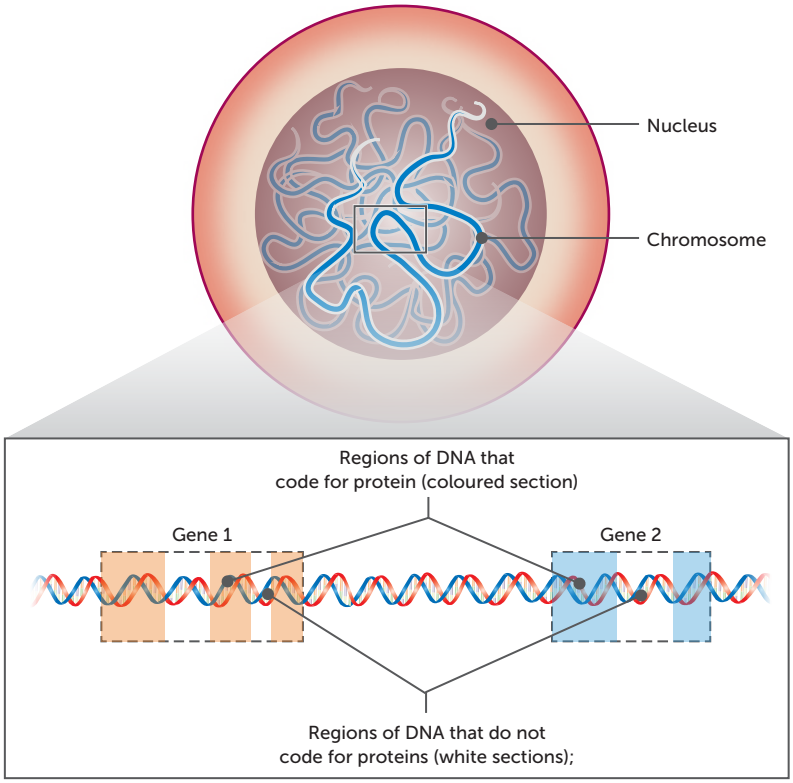


FIGURE 3.17 Coding versus non-coding DNA in a eukaryotic cell: 75% of non-coding DNA occurs between genes. Introns occur within genes and they make up the remaining 25% of non-coding DNA.

The genetic code
Watch the animation. Describe how the code is stored.

The genetic code

The genetic code is the term used for the way that the four nitrogenous bases of DNA, adenine, thymine, guanine and cytosine, are ordered. The base order is 'read' by **cellular machinery** and turned into a protein via a process called **protein synthesis**. Cellular machinery consist of 'biological machines' that work to manufacture a biological molecule. The **transcription** machinery includes RNA polymerase and binding factors/proteins.

The **translation** machine is the **ribosome**. In the genetic code, each set of three DNA nucleotides in a row counts as a **triplet** and codes for an **mRNA (messenger RNA)** triplet called a **codon**. The mRNA codon (three nucleotides) is

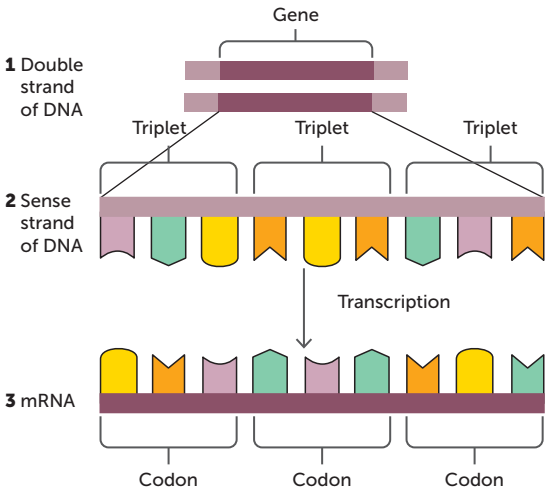


FIGURE 3.18 The genetic code is a base triplet code.

again read by cellular machinery and is translated into a single amino acid. Each sequence of three nucleotides codes for an amino acid. Given that some proteins are made up of hundreds of amino acids, the code that would make one protein could have hundreds, sometimes even thousands, of triplets contained in it.

Key concept

The genome sequence consists of coding DNA (genes) and non-coding DNA. Three coding DNA nucleotides make a triplet, which matches an mRNA codon.

Question set 3.4

REMEMBERING

1 Define:
   - **a** genome sequence
   - **b** coding DNA
   - **c** non-coding DNA.

2 State the link between an organism's genotype and phenotype.

UNDERSTANDING

3 Differentiate between a DNA triplet and a codon.

ANALYSING

4 Why are nucleotide sequences read in threes?

3.5 PROTEIN SYNTHESIS

Proteins are essential to the structure and function of cells, and thus also to the structure and function of organisms. Protein synthesis is the process of making new proteins from the genetic information encoded in DNA. There are two main processes that facilitate the flow of information from gene to protein: transcription and translation. Transcription is the synthesis of mRNA using the stored DNA code. The synthesised mRNA is a chain of RNA nucleotides complementary to the DNA strand, except uracil (rather than thymine) is the base pair of adenine in RNA. Translation is the synthesis of a polypeptide using the information in the mRNA. The RNA nucleotide code is translated into an amino acid sequence.

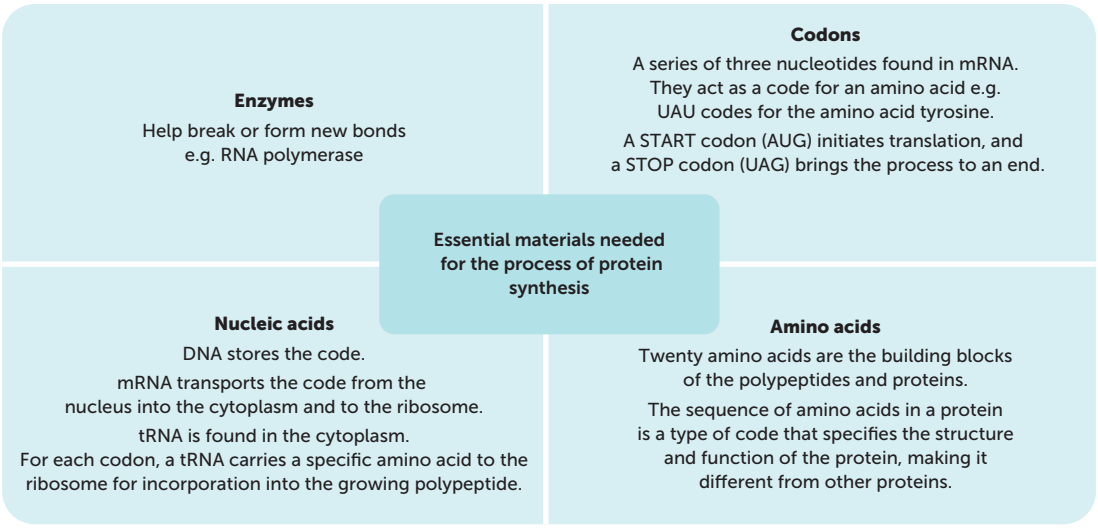


FIGURE 3.19 Major materials required in protein synthesis and their roles

Genes are found in chromosomes in cells. They are sequences of DNA that code for a protein. It is only during cell division that the DNA can leave the nucleus of a eukaryotic cell. Otherwise, it remains there, ready for future cell division (mitosis or meiosis). Thus, the DNA code (genes) must be transcribed into messenger RNA (mRNA) while still inside the nucleus. The mRNA can fit through the **nuclear pores** because it is a short, single-stranded molecule. Therefore, the mRNA can carry the code of instructions

to the ribosome, where translation can take place. The ribosome binds to the mRNA. Each codon attracts the corresponding **anticodon** that forms part of a **tRNA (transfer RNA)** molecule. The tRNA molecule carries the amino acid that is specific to the codon. As one codon at a time moves into and is read by the ribosome, successive tRNAs transport amino acids to it, translating the code by dropping off amino acids in a sequence that matches the sequence of codons. Gradually, a polypeptide is produced (a string of amino acids, joined by **peptide bonds**). The polypeptide can detach and fold to form a protein by itself, or attach to other polypeptides and then fold to form a protein.

Transcription and translation in prokaryotes

In a prokaryotic cell (a cell that lacks membrane-bound **organelles**, including nuclei), the chromosome is generally in the form of a closed circle that is not wrapped around histone proteins. It is found in the region of the cell known as the nucleoid. In addition to the single chromosome, bacteria may contain **plasmids**, which are small rings of DNA. Plasmids code for traits but are not essential to the survival of the cell (although they may aid in its survival). Transcription begins when a section of the double-stranded chromosome is separated and enzymes synthesise an mRNA product complementary to the template strand. In prokaryotes, transcription and translation are simultaneous; that is, translation begins while the mRNA is still being synthesised, during transcription. Numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In contrast, a eukaryotic cell performs transcription in the nucleus, and translation in the cytoplasm.

Question set 3.5a

REMEMBERING

1 Describe why protein synthesis is needed.

2 State the two main processes involved in protein synthesis.

UNDERSTANDING

3 Describe four essential materials required for protein synthesis and include the function of each in the synthesis of a protein.

ANALYSING

4 In eukaryotes, translation follows transcription. Differentiate between transcription and translation in prokaryotes and eukaryotes, and state why it may be simpler in prokaryotes.

Transcription in eukaryotes

Transcription, a process that produces mRNA from DNA, occurs in the nucleus in eukaryotes. During transcription, one section of DNA, called a gene, is unwound and separated ready for copying. RNA polymerase moves step by step along the DNA molecule, separating the two strands. Only the template strand is copied. The **template strand** is also known as the **antisense** or **non-coding strand**. The other strand is known as the **non-template, sense** or **coding strand**. The coding strand has the same code as the mRNA, except in RNA uracil replaces thymine. The sequence of the DNA nucleotides determines the sequence of the RNA nucleotides, because RNA polymerase attaches the RNA nucleotide that is complementary to each DNA base. The complementary pairs are added according to the base-pair rules, shown in Table 3.3.

TABLE 3.3 The complementary base pairs attach during transcription according to base pair rules.

DNA NITROGENOUS BASE	COMPLEMENTARY RNA NITROGENOUS BASE IN THE RNA NUCLEOTIDE
Adenine	Uracil
Thymine	Adenine
Cytosine	Guanine
Guanine	Cytosine

A **promoter** attaches to help the DNA template strand to locally separate from the non-template strand, initiating transcription. RNA polymerase binds to the DNA to get ready to start synthesis. RNA polymerase synthesises the mRNA in a 5' to 3' direction, anti-parallel to the template strand. The mRNA nucleotide triplets are called codons. The codons are complementary to the template strand but almost identical to the non-template/coding strand, except for uracil replacing thymine. After the RNA polymerase enables elongation of the strand, the mRNA molecule detaches as **pre-mRNA**. Pre-mRNA requires processing before it exits the nucleus via the nuclear pore. Stretches of non-coding DNA (known as introns) are removed and the remaining stretches of DNA (known as exons) join to form **mature mRNA**.

SCIENTIFIC LITERACY

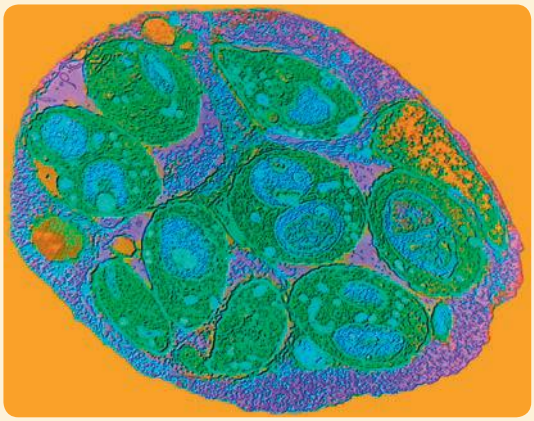
Introns and exons

As part of the normal process of generating proteins from genes stored in DNA, the code for constructing a particular protein is passed from stored DNA to a form that is transportable known as messenger RNA (mRNA). The strip of mRNA that is first formed when the DNA code is copied has excess baggage. In most eukaryotes, the mRNA initially carries the instructions for making a protein, but also carries extra nucleotides that are not needed. The unrefined mRNA is called pre-mRNA.

Before the mRNA can leave the cell nucleus, non-coding regions called introns are cut out in a process called (pre-)mRNA splicing. The remaining exons join together as the final set of refined instructions, ready to move out of the nucleus via a nuclear pore. The refined mRNA is called mature mRNA. It performs the function of carrying the code to the translation site, where proteins are built one amino acid at a time according to the code.

The discovery of mRNA splicing in the late 1970s was simultaneous with the revelation that a single species of pre-mRNA could be spliced in different ways, creating multiple, distinct mature mRNAs. This is now known as 'alternative splicing'. The various mature mRNAs contain different combinations of exons. The different combinations give rise to different proteins.

Scientists at the American Society for Microbiology have studied alternative splicing in Apicomplexan parasites. Apicomplexan parasites are pathogens (organisms that cause an infectious disease) found in humans and domestic animals. These parasites have also been reported by CSIRO as being parasitic on Australian reptiles, mammals such as the echidna, and the green tree frog. Funding for research into parasites of Australian wildlife is sparse, and anti-parasitic drugs have been hard to develop. Perturbation (interruption) of alternative splicing has been found to be detrimental to these parasites, making it a worthy drug target to pursue if we wish to reduce disease in humans and domestic animals.



Science Photo Library/ALAIN POL/ISM

FIGURE 3.20 *Toxoplasma gondii*, a species of Apicomplexan parasite

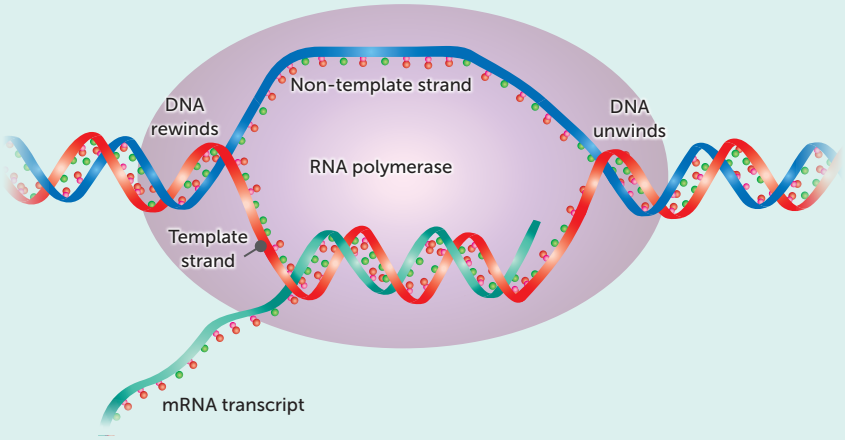
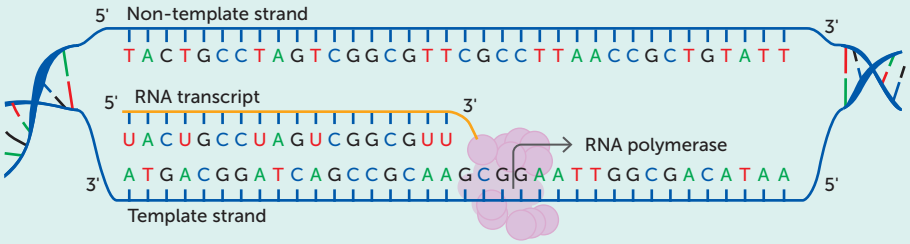
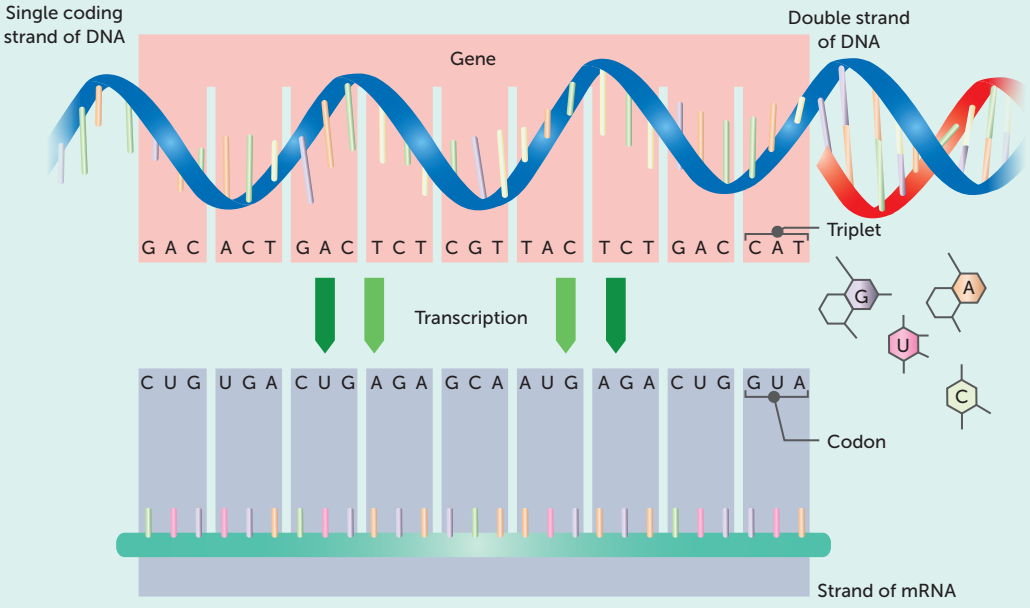
Questions

1. Describe pre-mRNA splicing.
2. Explain the rationale for spending money on research into parasites of Australian wildlife.
3. Describe the relationship between studying alternative splicing and anti-parasitic drugs.

mRNA splicing
Watch this animation to help you understand pre-mRNA splicing:

9780170452922

TABLE 3.4 Summary of transcription

STEP	VISUAL AID
Preliminary information Only one of the two strands of DNA is used for transcription: the template strand (also known as the non-coding strand or the antisense strand). **1** RNA polymerase binds to a promoter region. It breaks the weak hydrogen bonds joining the complementary nucleotides and unzips (unwinds) a portion of the double helix.	 **FIGURE 3.21** RNA polymerase binds to a promoter region and an area of one gene on the DNA molecule becomes unzipped, beginning transcription.
2 Moving along the template DNA strand in a 3' to 5' direction, the RNA polymerase adds free-floating nucleotides to the growing mRNA sequence according to the complementary base-pair rules, but in RNA uracil pairs with adenine. The new strand of mRNA is synthesised in a 5' to 3' direction.	 **FIGURE 3.22** mRNA is synthesised in a 5' to 3' direction by RNA polymerase.
3 The DNA bases are in triplets, and the complementary mRNA triplets produced are called codons. The process continues until there is a termination signal and the pre-mRNA is released.	 **FIGURE 3.23** The mRNA produced contains codons that are complementary to the DNA triplets.

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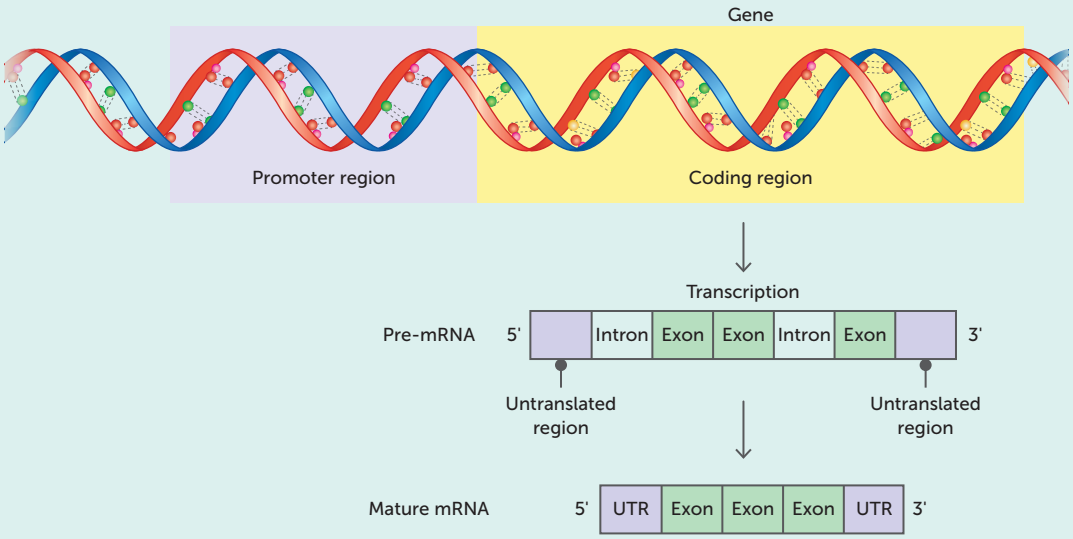
STEP	VISUAL AID
4 Only the coding region (gene) of DNA is transcribed. Pre-mRNA consists of introns and exons. The introns are removed and the exons are joined to create mature mRNA. Mature mRNA then exits the nucleus via a nuclear pore.	

FIGURE 3.24 The coding region of DNA is transcribed into pre-mRNA, which is then processed to make mature mRNA.

Key concept

Protein synthesis in eukaryotes includes the process of transcription. Transcription occurs in the nucleus and is the process of transcribing the code from DNA into a smaller molecule called mRNA, which can then leave the nucleus.

Question set 3.5b

REMEMBERING

1 Explain the purpose of transcription.
2 State the site for eukaryotic transcription.

UNDERSTANDING

3 Differentiate between a DNA triplet and an mRNA codon.

APPLYING

4 Apply what you have learned about coding and non-coding DNA to differentiate between introns and exons.

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3D animation of DNA to RNA to protein

Translation in eukaryotes

Protein synthesis involves both transcription and translation. These processes enable genetic information to flow from DNA to mRNA to protein. Transcription is the process of synthesising a copy of the DNA code in the form of mRNA. Translation is the RNA-directed synthesis of a polypeptide.

Ribosomes facilitate the interaction of mRNA and tRNA to position and connect a specific sequence of amino acids. Ribosomes are mostly composed of ribosomal RNA (rRNA), which is non-coding.

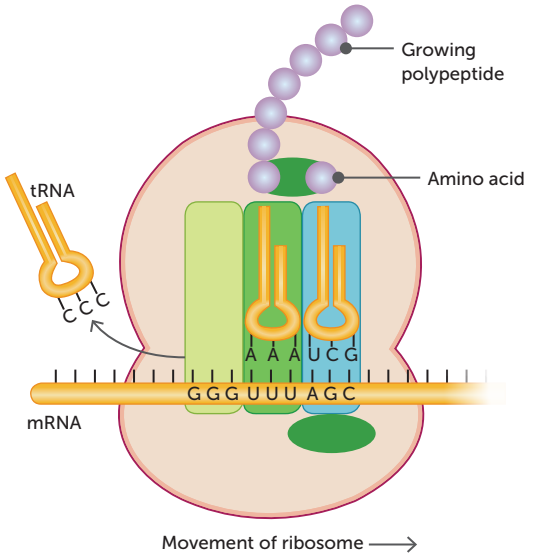

FIGURE 3.25 Ribosomes read codons to construct polypeptides.

Process of translation

After mRNA moves out from the nucleus through a nuclear pore, it enters the cytoplasm and travels to a ribosome, where it will be read and translated. The translation process can be divided into three main stages: initiation, elongation and termination.

Initiation

A ribosome binds to a molecule of mRNA. It 'reads' the mRNA nucleotides in threes. A group of three consecutive nucleotides is called a codon. A special codon, AUG, is the start codon and codes for the amino acid methionine. It signals the start of translation and the beginning of a polypeptide chain. The tRNA molecule that contains the anticodon UAC is attracted to the start codon and pairs with it. This tRNA molecule brings with it the amino acid methionine. At initiation, two codons enter and are bound to the ribosome, but after that only one codon enters and is translated at a time.

Elongation

A tRNA molecule, which includes in its sequence an anticodon, is attracted to the corresponding codon on the mRNA due to complementary base pairing. Each tRNA molecule carries an amino acid specified by the codon that it pairs with. As one codon is read and exits the ribosome, another one slides in to be read. tRNAs transfer the amino acids to the mRNA–ribosomal complex in the order specified by the codons of the mRNA. The ribosomes catalyse the formation of covalent peptide bonds between adjacent amino acids. The mRNA is moved through the ribosome in one direction only. Once a tRNA molecule has dropped off its amino acid, it will return to the cytoplasm to reload with the same type of amino acid. Note that the tRNA is not used up during translation, and some amino acids are coded for by more than one codon.

From DNA to protein
Watch this short video on protein synthesis.

Termination

Elongation continues until a stop codon in the mRNA enters the ribosome. The nucleotide base triplets UAG, UAA and UGA do not code for an amino acid. Instead, any one of them acts as a signal to stop translation. The polypeptide is then released and the mRNA leaves the ribosome. Once removed, the polypeptide may fold (or join with another polypeptide to fold) to become a structural or functional protein. The protein will either be used in the cell it was formed in or be transported out of the cell for use elsewhere. Note that the tRNA is not used up during the translation process, and some amino acids are coded for by more than one codon.

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TABLE 3.5 Summary of translation

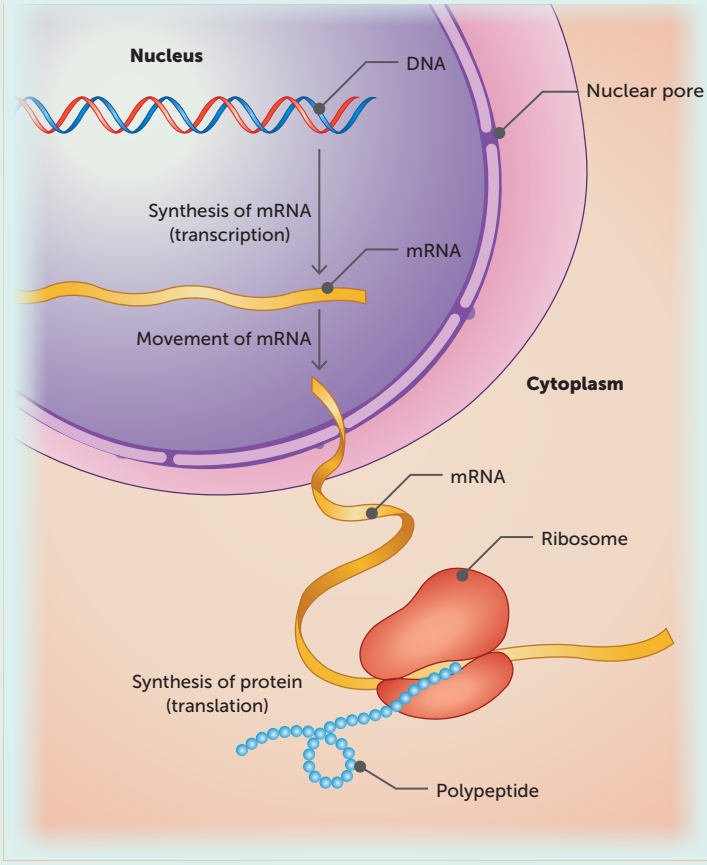
STEP	VISUAL AID
1 The short, single-stranded mRNA leaves the nucleus via a nuclear pore to bind with a ribosome in the cytoplasm. A subunit of the ribosome binds to mRNA to begin protein synthesis.	

FIGURE 3.26 mRNA leaves the nucleus and binds with a ribosome in the cytoplasm.

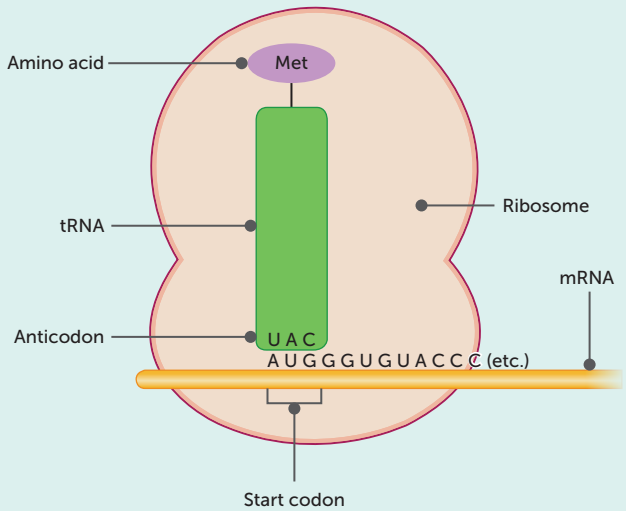
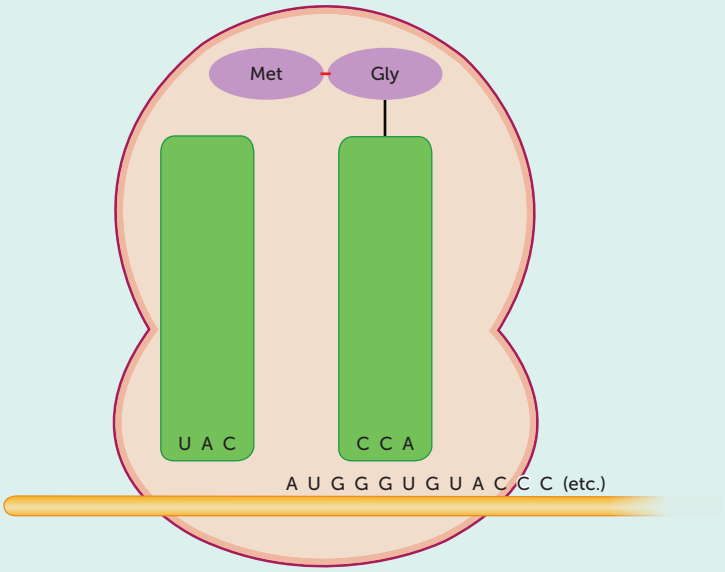
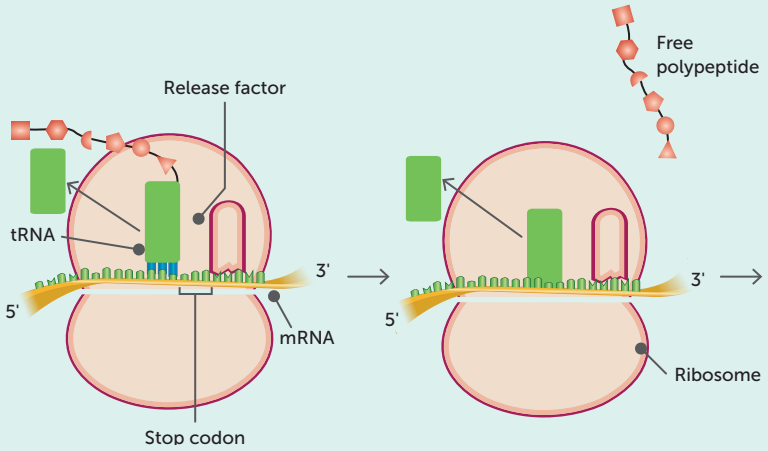
2 A start codon (AUG) in the mRNA molecule signals for a tRNA molecule with the complementary anticodon (UAC) to arrive for base pairing. The tRNA molecule carries the first amino acid, methionine.	

FIGURE 3.27 The start codon in mRNA is usually AUG.

STEP	VISUAL AID
3 The next codon attracts a new tRNA molecule with the corresponding anticodon and amino acid attached. One tRNA molecule at a time, with its specific amino acid attached, moves into the ribosome while the other, now minus its amino acid, leaves the ribosome. After each codon partners with an anticodon, the amino acid is removed from the tRNA and is joined to a growing amino acid chain by a covalent peptide bond. The ribosome 'reads' one codon at a time.	 **FIGURE 3.28** Amino acids are attached one at a time and according to the order of the codons.
4 When a stop codon appears, elongation ceases and the polypeptide is released from the ribosome. The ribosome separates from the mRNA.	 **FIGURE 3.29** The polypeptide is formed and released.

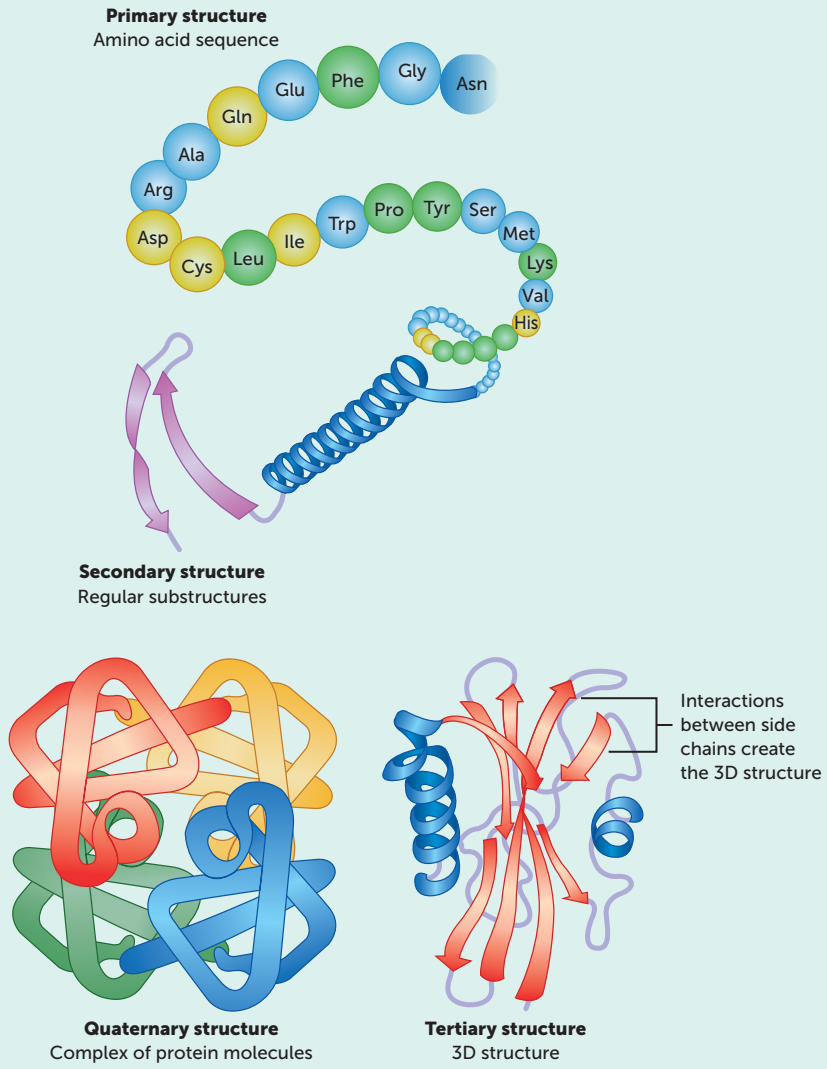
STEP	VISUAL AID
5 The polypeptide either folds to form a protein or joins with another polypeptide to fold to become a 3D protein ready to perform a function. Primary, secondary, tertiary and quaternary describe different levels of protein structure, from simple to complex.	

FIGURE 3.30 Protein synthesis is complete.

Key concept

Protein synthesis in eukaryotes includes translation. Translation occurs at a ribosome in the cytoplasm, and uses the code in mRNA to produce a sequence of amino acids called a polypeptide.

Question set 3.5c

REMEMBERING

1. Define polypeptide.
2. State the enzyme(s) involved in separating the two strands of DNA and synthesising an mRNA strand.

UNDERSTANDING

3. Describe the relationship of tRNAs to mRNA and amino acids.

CREATING

4. Create a flow diagram showing the summarised steps of transcription and translation.

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3.6 PROTEINS

Proteins are built of their basic units or monomers (known as amino acids) and are essential to cell structure and functioning. Some proteins are quite rigid, such as collagen (which plays a structural role in the connective tissue of mammals). Other types of protein, such as enzymes, perform functional tasks. Enzymes (e.g. lipase and trypsin) are catalysts that increase the rate of virtually all of the chemical reactions within cells. The protein shape at the active site of an enzyme determines the specificity of the enzyme: only specific enzymes can fit with specific substrates. In addition to providing mechanical support and functioning as catalysts, proteins transport and store other molecules (such as oxygen), provide immune protection, generate movement, transmit nerve impulses, and control growth and differentiation.

A protein's structure is vital to its function. A slight change in structure can alter the function of a protein to the extent that cell death may be triggered. Programmed cell death (**apoptosis**) is an important strategy for disposing of damaged or infected cells and those no longer needed in a multicellular organism.

Proteins are built from a selection of 20 different amino acids. The amino acids are linked together by peptide bonds to form polypeptide chains, which fold and/or are modified to form the protein. The sequence of amino acids in a polypeptide is determined by the sequence of mRNA codons in a strand of mRNA. If the sequence of codons is known, the sequence of amino acids can be determined from an **amino acid table** (also known as a **codon table**, Figure 3.31. A codon table is a translation table that identifies the amino acids that correspond to the mRNA codons. To find the amino acid coded for by an mRNA codon, look for the three nitrogenous base letters in the table. There are 64 possible base triplets (4 × 4 × 4), and three of these are stop codons that signal for translation to stop.

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

Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartic acid
Cys = cysteine
Gln = glutamine
Glu = glutamic acid
Gly = glycine
His = histidine
Ile = isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = tyrosine
Val = valine

FIGURE 3.31 The genetic code is shown by a codon table. The mRNA codons correspond to the 20 amino acids used to build polypeptides during translation on the ribosomes. Three codons act as stop codons, and (under certain conditions) the codon AUG initiates protein synthesis.

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The genetic code for keratin, a protein building block for hair, is transcribed and translated by specialist cells underneath growing hair. The following sequence is part of the mRNA molecule that is transcribed from the gene for keratin: AUGUCUCGUGAAUUUUCC.

To determine the sequence of amino acids, divide the nucleotides from the gene into sets of three.

AUG UCU CGU GAA UUU UCC

Then use the codon table (Figure 3.31) to translate the code. The first codon (AUG) is a start codon that codes for an amino acid called methionine. Continuing along the gene, the entire sequence for this section of the code is:

methionine–serine–arginine–glutamic acid–phenylalanine–serine

Question set 3.6

REMEMBERING

1. Define protein.
2. State two of the main types of proteins needed in an organism.
3. Give an example of each of the two types of protein in your answer to the previous question, and describe their functions.

UNDERSTANDING

4. Compare and contrast a codon and anticodon.

ANALYSING

5. Use the codon table (Figure 3.31) to determine the chain of amino acids for the following DNA code: UACAGAGCACUUAAAAGG.

The koala's DNA has been sequenced

CASE STUDY

Koala populations in NSW and Queensland dropped by 42% between 1990 and 2010, according to the federal Threatened Species Scientific Committee. Reasons for the decline included chlamydia (an infectious disease), bushfires (destroying their habitat), wild dogs and climate change. Koalas are particularly vulnerable to climate change because they are heavily reliant on trees for their homes and food. The severe population declines experienced by koalas have prompted WWF-Australia and other groups to nominate the koala for to have its listing changed from vulnerable to endangered.

A team of Australian and international scientists, led by Adjunct Professor and Australian Museum Research Institute director Rebecca Johnson (pictured) and Professor Katherine Belov at the University of Sydney, completed the world-first full **sequencing** of the koala genome in 2018. The entire sequence of nucleotides found in a koala's DNA was recorded.

The Australian-led Koala Genome Consortium of 54 scientists from 29

Fairfax Photos/Jessica Hromas

FIGURE 3.32 Researchers such as Rebecca Johnson aim to help koala conservation using genomic studies.

institutions across seven countries sequenced the more than 3.4 billion base pairs and more than 26 000 genes in the koala genome, which makes it slightly larger than the human genome.

You may be wondering how this helps the plight of the Australian koala? Koala joeys are born after 35 days of gestation, when they are the size of a jelly bean. While they are in their mother's pouch, they are protected by antimicrobial peptides in her milk. However, when they are weaned they no longer have this protection. They are then susceptible to a bacterial infection called chlamydia. Sequencing the genome has allowed scientists to characterise the architecture of their immune system and to identify genes that play a role in resistance and susceptibility to chlamydia. The DNA data can be used to help develop vaccines, manage koala populations and ultimately help with their long-term survival.

Some of the 26 000 koala genes identified in the koala genome project help explain the koala's extraordinary ability to survive almost exclusively on eucalypt leaves, a diet high in toxins. Researchers found an abundance of genes for bitter taste receptors (which would allow koalas to identify the least toxic leaves), as well as genes that code for proteins that help detoxify the poisonous substances.

Questions

1. Propose a logical reason why it took so long for the nucleotides in a koala's DNA to be sequenced (i.e. the order of nucleotides to be determined)?
2. List four changes in the koala's environment that makes them vulnerable today.
3. Explain how our knowledge of the sequence of nucleotides (the genome) may be useful for the koala's long-term survival.

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CHAPTER 3 ACTIVITY AND INVESTIGATION

3.1 ACTIVITY

The genetic code

The nucleus of eukaryotic cells is packed with DNA, the molecule that is the template for all the proteins produced by the cell. Ribosomes, the sites of protein synthesis, are found in the cytoplasm, outside the membrane of the nucleus. DNA is unable to move through the nuclear membrane, so in order to produce a protein, a message must be sent from the nuclear DNA to the ribosome.

To do this, two processes take place:

1. transcription of the message from the DNA into an mRNA molecule
2. translation of the message in the mRNA into a specific amino acid sequence at the ribosome.

The molecule of mRNA is transcribed from the template DNA strand using the complementary sequences. However, the thymine present in DNA is replaced with uracil in RNA. The complementary pairs in RNA are A–U and G–C.

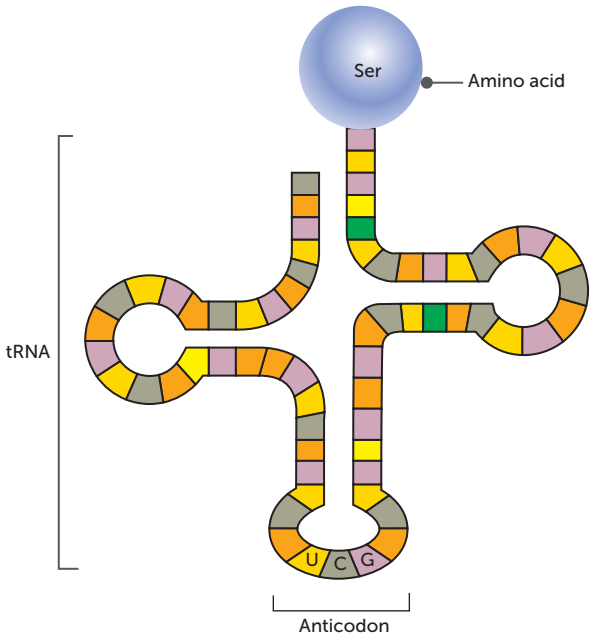

FIGURE 3.33 The amino acid serine being carried by a tRNA molecule

Translation of the mRNA message occurs at ribosomes, where the sequence of nitrogenous bases in the mRNA is read in groups of three called codons. Each tRNA molecule contains an anticodon that is complementary to the codon of the mRNA, and each tRNA binds a specific amino acid. The tRNA molecules bring their amino acids to the ribosome, where the amino acids are bonded together, forming a long chain in a specific sequence according to the sequence of the mRNA being translated.

Aim

To simulate the production of a protein from a sequence of DNA

You will need

- A3 paper
- Coloured pencils

What to do

The sequence of nucleotides that will be used in this activity codes for the enzyme lysozyme.

ATGACCCATGCGTTAGGC

Refer to the genetic code in Figure 3.31 (page 74), the sequence of nucleotides in mRNA that codes for each of the specific amino acids needed in the synthesis of proteins in nearly all organisms.

1. Divide a piece of A3 paper into six sections.
2. In the first section of the A3 paper, draw a nucleus containing the DNA template for the complementary strand, using the sequence provided above.
3. In the second section of the A3 paper, show the process of transcription of the template DNA into mRNA.
4. In the third section of the A3 paper, show the movement of mRNA out of the nucleus.

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5 In the fourth section of the A3 paper, show the process of translation of the message.
6 In the fifth section of the A3 paper, describe in words, the process of protein synthesis that you have just illustrated in diagrams.

What did you discover?

1 Describe in your own words the processes of transcription and translation. Include an explanation of where in the cell each process takes place and which other molecules are involved in each process. Explain why the cell needs each process for sustaining life.
2 Describe how the hydrogen bonds are re-joined between complementary DNA nucleotides during transcription.
3 State the sequence of the nucleotides in the transcribed mRNA sequence of lysozyme.
4 DNA is transcribed to make mRNA, but not all transcribed DNA contains codes for a protein. These non-coding sections get broken down to make nucleotides for re-use in the nucleus. What is the name for these sections?
5 State the anticodon sequence for the lysozyme protein. Explain the importance of anticodons in these processes.
6 State the final amino acid sequence coded for by the length of DNA you are working with.
7 Explain the role of uracil in the process of transcription.

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3.1 INVESTIGATION

Strawberry DNA extraction

Background

Strawberries have eight sets of chromosomes, making them octoploid (along with pansies, dahlias and sugar cane). Strawberries are a very effective model for DNA extraction, because their juice provides a pink solution in which, when treated, the white strands of DNA can be clearly observed.

Aim

To use restriction enzymes to cut DNA into fragments of varying length

Time requirement

30 mins

Materials

- 3 strawberries
- 1 resealable plastic storage bag
- 10 mL DNA extraction buffer
- 5 mL protease enzyme
- 1 inoculation loop
- Test tube
- 2 plastic pipettes
- 1.5 mL centrifuge tube
- Filter paper
- Glass funnel
- 5 mL ice-cold 95% ethanol
- Personal protective equipment, such as lab coats, safety glasses, gloves

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Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Ethanol is highly flammable.	Store and use away from ignition sources. Do not heat in a container over an open flame; use a water bath that is spark proof.
Protease enzyme may cause skin irritation.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves may pose an allergy risk.	Use a type of glove that has no allergy risk and is suitable for the chemicals being used.
Strawberries may pose an allergy risk.	Never allow any food to be eaten in class. Check whether anyone has a known allergy.

Procedure

1. Place three strawberries in a plastic storage bag and re-seal.
2. Squeeze the strawberries in the bag with your fingers until it they are lightly crushed.
3. Open the storage bag and add 10 mL of the DNA extraction buffer.
4. Re-seal the bag and crush the contents again, using your hands to mix the ingredients. Continue until a thick juice is produced.
5. Using a plastic pipette, add 5 mL of protease enzyme and hand mix through for one minute.
6. Filter the strawberry juice into a test tube. To do this, place filter paper in a glass funnel over the test tube. Pour the strawberry juice into the filter paper. A clear, pulp-free juice will filter through into the test tube.
7. Remove the filtering apparatus and slowly pour approximately 5 mL of cold ethanol or 70–90% isopropyl alcohol into the test tube to cover the strawberry juice solution. Do not agitate the solution. The ethanol should sit separately on top of the strawberry solution.
8. The cell walls will break down and white strands of strawberry DNA will become visible in the ethanol layer as the DNA is extracted from within the nuclei. The strands will look like very fine spider webs.
9. Use an inoculation loop to 'spool' strands of DNA and observe them more closely. Alternatively, hold the test tube at eye level and use a pipette to draw up the DNA strands in the top layer of the fluid.
10. Transfer the DNA strands to a centrifuge tube for further examination.

Results

Describe what you see.

Discussion

1. What roles did the detergent, protease enzyme, ethanol and salt have in the process of DNA extraction? Many of the foods we consume contain DNA. Explain why ingesting DNA from other plants and organisms does not cause us harm or alter our DNA.
2. What is the function of DNA?
3. Where is DNA located within a cell?
4. Draw a diagram of DNA. Include five pairs of nucleotide bases and label the hydrogen bonds between these bases.
5. Explain why the ability to remove DNA from cells is important to scientists.
6. Why does DNA go up towards the surface when the ethanol is added?
7. Summarise your findings and include a flow chart detailing the steps taken to release DNA from the strawberry cells.

Taking it further

Perform another DNA extraction using alternative plants, such as banana, kiwifruit and wheatgerm. Following the same procedure, compare the results of the DNA extraction between the various plant samples.

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WS
Chapter 3 Activity sheet

CHAPTER 3 SUMMARY

- Rosalind Franklin, James Watson, Francis Crick and Maurice Wilkins are credited with the discovery of the structure of DNA in 1953.
- DNA is bound to proteins, forming linear chromosomes in the nucleus of eukaryotes, but unbound and circular in the mitochondria and chloroplasts.
- DNA also exists in an unbound, circular form in the nucleoid region of the cytosol of prokaryotes.
- DNA is composed of four different types of nucleotides; each nucleotide has a deoxyribose sugar, a phosphate group and a nitrogenous base.
- The two strands of a DNA double helix are linked by weak hydrogen bonds between the complementary bases: adenine (A) pairs with thymine (T), and cytosine (C) pairs with guanine (G).
- Like DNA, RNA is composed of nucleotides; however, in RNA each nucleotide has a ribose sugar, and it contains uracil (U) instead of thymine (T).
- The sugar–phosphate backbone of the nucleotides is arranged in a 5' to 3' direction.
- DNA replicates by a semi-conservative mechanism, whereby one of the strands in the newly formed molecule is new and the other is from the original strand.
- The enzymes helicase, polymerase and ligase facilitate DNA replication.
- All of the DNA in the cell of an organism is referred to as its genome or genetic code, and the nucleotides are grouped into threes (triplets).
- DNA includes coding and non-coding sections. Coding DNA contains instructions for the production of a protein.
- Protein synthesis involves transcription of a gene into messenger RNA (mRNA) in the nucleus, and translation of the mRNA code into an amino acid sequence at the ribosome.
- A chain of amino acids is called a polypeptide. At the conclusion of protein synthesis, a polypeptide will fold or be modified to become an active protein.
- Protein structure determines function, and can be described as primary, secondary, tertiary or quaternary.
- Proteins are essential for an organism's survival. Two of the main protein types are structural proteins (e.g. collagen) and enzymes (e.g. lipase).

CHAPTER 3 GLOSSARY

5' to 3' The direction of synthesis on a nucleotide strand

Amino acid An organic compound that is a building block within a polypeptide or protein

Amino acid table See **codon table**

Anticodon A set of three consecutive nucleotides that is part of a tRNA molecule and is complementary to a codon; the three nucleotides consist of any of the four bases adenine, uracil, guanine or cytosine

Apoptosis A programmed series of events that leads to cell death (as a result of the dismantling of the internal contents of the cell by various enzymes, including caspases)

Cellular machinery 'Biological machines' that work to manufacture a biological molecule; e.g. transcription machinery includes RNA polymerase and binding factors or proteins; the translation machine is the ribosome

Chromosome A structure composed of DNA and protein that contains linear arrays of genes carrying genetic information; prokaryotes generally have one circular chromosome, whereas eukaryotes have a number of linear chromosomes

Coding DNA The sections of DNA that code for a protein; they contain instructions that determine the order of the codons in the mRNA, which in turn determines the order of the amino acids in a polypeptide or protein

Codon A set of three consecutive nucleotides found in a DNA or an mRNA molecule; it

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carries codes for a specific amino acid; the three nucleotides consist of any of the four bases adenine, thymine, guanine and cytosine in the case of DNA, or adenine, uracil, guanine and cytosine in the case of mRNA

Codon table (amino acid table) A translation table for determining the amino acid coded for by an mRNA codon; the three nitrogenous base letters can be looked up in the table to find the name of the specified amino acid

Complementary base pairing The phenomenon whereby guanine always hydrogen bonds with cytosine and adenine always hydrogen bonds with thymine; guanine and cytosine share three hydrogen bonds, and adenine and thymine share two hydrogen bonds; the complementary pairing enables the helical structure of DNA to form

DNA (deoxyribonucleic acid) The information-containing molecule present in all living things that contains the instructions, written in a chemical code, for the production of proteins by the cell; the information it contains is sufficient for the making and maintaining the organism; in addition, DNA is the genetic material that passes this information on to the next generation

DNA helicase An enzyme that helps the two strands of the DNA double helix unwind and separate

DNA ligase An enzyme used to catalyse the formation of a bond between two pieces of DNA

DNA polymerase A member of a class of enzymes found in all living things, that synthesises new strands of DNA based on a template strand and according to complementary base-pair rules; DNA polymerases are important tools in biotechnology because they are capable of making exact copies of fragments of DNA, enabling efficient and accurate amplification of DNA templates

DNA replication The process a DNA molecule undergoes to make a complete and identical copy of itself, readying a cell for cell division; it is a semi-conservative process, and the two daughter molecules contain exact copies of the genetic material in the parent molecule

Double helix The structure formed by double-stranded molecules of nucleic acids such as DNA; two linear strands that run opposite to each other and twist together

Enzyme A reusable, biological catalyst that lowers the activation energy of a chemical reaction, making it proceed faster; it is a protein that is sensitive to factors such as temperature and pH

Gene A unit of heredity that transmits information from one generation to the next; a segment of DNA that codes for a polypeptide

Genetic code The term used for the way that the four nitrogenous bases of DNA (adenine, thymine, guanine and cytosine) are ordered and contain information to direct the production of specific proteins

Genome All of the genetic material contained in an organism or a cell; it includes the sequence of the DNA in the chromosomes within the nucleus, mitochondria and chloroplasts

Genome sequence The sequence of consecutive DNA 'letters' spanning all the chromosomes of a cell from start to finish

Genomics The study of the genome – how genes interact with one another, the environment and the resultant proteins produced; knowledge of an organism's entire DNA sequence

Heredity The study of inheritance, the genetic transmission of characteristics from one generation to another

Lagging strand The DNA strand that is synthesised discontinuously in small fragments, called Okazaki fragments, in a 5' to 3' direction

Leading strand The DNA strand that is synthesised continuously in a 5' to 3' direction

Mature mRNA mRNA that has been processed after transcription; non-coding introns have been removed and the remaining exons joined

mRNA (messenger RNA) The RNA molecule that carries the information from a gene to a ribosome for translation into a polypeptide; in eukaryotes it carries the message from the DNA in the nucleus out through a nuclear pore to a ribosome in the cytoplasm

Nitrogenous base A structural component of the nucleotides that make up DNA or RNA

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Non-coding DNA All of the DNA sequences within a genome that are not found within mRNA-coding exons; examples include introns, promoters and enhancers of genes; they have no known function

Non-template/sense/coding strand The coding strand is also known as the sense strand; this strand has the same code as the mRNA strand, except uracil replaces thymine; it is not read during transcription

Nuclear pore A small opening in the nuclear membrane through which relatively small single-stranded molecules such as mRNA can fit

Nucleoid The region within a prokaryotic cell that contains the genetic material

Nucleotide The basic building block of nucleic acids (DNA and RNA); nucleotides are linked together by phosphodiester bonds; each nucleotide is made up of a five-carbon sugar, a phosphate group and a nitrogenous base

Okazaki fragment A short fragment of DNA synthesised during DNA replication; multiple fragments are joined together to make the lagging strand during replication

Organelle A specialised part of a cell that has its own specific function; a 'little organ'

Peptide bond A covalent bond that links amino acids in a polypeptide

Phosphodiester bond A covalent bond that links a 3' carbon in one sugar to a 5' carbon in another sugar in DNA and RNA; it consists of a phosphate group, its covalent ester bond with the 3' carbon and its covalent ester bond with the 5' carbon; this bond connects nucleotides, which form the backbone of a DNA or RNA chain

Plasmid A small, circular piece of DNA, found in bacteria, that is able to replicate independently of the cell's chromosome; engineered plasmids may carry antibiotic-resistance markers

Polypeptide A string of amino acids, joined by peptide bonds

Pre-mRNA The strand of precursor mRNA that is first produced after transcription of a gene; it contains introns and exons (from non-coding and coding DNA, respectively); it is processed to become mature mRNA

Promoter A relatively short nucleotide sequence in the DNA of a gene that attaches RNA polymerase to the start of the strand to begin synthesis of RNA during transcription

Protein A type of essential biological macromolecule; the structure of each protein is vital to its function; proteins are made of one or more folded and modified polypeptides

Protein synthesis The process whereby cells produce proteins from instructions encoded in genes found in the coding section of the cell's DNA; the process can be divided into two major steps: transcription and translation

Replication fork The junction between the unwound single strands of DNA and the intact double helix during DNA replication

Ribosome An organelle found in prokaryotes and eukaryotes; it facilitates the interaction of mRNA and tRNA in transporting and connecting specific sequences of amino acids into polypeptides (translation); it is mostly composed of rRNA and can be found attached to endoplasmic reticulum or alone in the cytosol of a cell

RNA (ribonucleic acid) A molecule consisting of a single strand of nucleotides; it plays an essential role in protein synthesis (as messenger RNA or transfer RNA) and as a structural component of ribosomes

Semi-conservative replication The production of two new DNA double-helix molecules, each consisting of one parental strand and one daughter strand

Sequencing The process of determining the order of nucleotides in a strand of DNA

Template/antisense/non-coding strand The strand of DNA that is read by a polymerase enzyme in order to attach the complementary base pairs

Trait An inheritable characteristic; phenotype

Transcription The synthesis of mRNA in which the sequence of nucleotides is complementary to the sequence in the stored DNA code, except that in RNA, uracil replaces thymine

Translation The synthesis of a polypeptide using the information in mRNA; the RNA nucleotide code is translated into an amino acid sequence

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Triplet A set of three consecutive nucleotides in DNA; the three nucleotides may consist of any of the four possible nitrogenous bases: adenine, thymine, guanine or cytosine

tRNA (transfer RNA) An RNA molecule that contains an anticodon (complementary to an mRNA codon); it carries an amino acid (specified by the codon) to a ribosome during protein synthesis

CHAPTER 3 REVIEW QUESTIONS

Remembering

1 State the type of chemical bond that holds the two strands of a DNA double helix together.
2 Describe the structure of DNA, including at least eight different features.
3 Describe the composition of an organism's genome.

Understanding

4 Define enzyme, and differentiate between the enzymes used in DNA replication and those used in protein synthesis.
5 Explain how accumulated information led to our current knowledge of DNA structure.
6 Describe how DNA may contain coding and non-coding DNA.
7 Explain why each of the following statements is incorrect and rewrite it as a correct statement.
   a One strand of the DNA double-helix ladder is maternal and the other strand is paternal.
   b Different organisms have different types of DNA, so they are very different from one another.
8 Describe the effect of a protein's structure on its function.

Applying

9 State two similarities and two differences between transcription and DNA replication.
10 Explain how nucleotides join together to form a polynucleotide chain.
11 Differentiate between pre-mRNA and mature mRNA.
12 Explain how the weak hydrogen bonding between nucleotides (complementary base pairing) in a DNA molecule allows semi-conservative DNA replication to occur.
13 Four amino acids are linked together in a very short section of a collagen protein.
   Glycine–Proline–Proline–Alanine
   Use the amino acid table (Figure 3.31, page 74) to determine the mRNA and DNA genetic code for this short section of the protein.

Analysing

14 Write the full names of DNA and RNA and record their structural differences in a table.
15 Scientists from the Australian-led Koala Genome Consortium have sequenced the koala genome. Analysis of the data reveals there are more than 26 000 genes on the 16 chromosomes.
   a Name the nucleotide that would be present in approximately the same number as the adenine nucleotides sequenced.
   b Explain whether you would expect the sequence of DNA to be exactly the same in all members of the koala species.
   c Estimate how many chromosomes a baby koala would get from its mother.
   d State a koala's diploid number and its haploid number.

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Evaluating

16 'DNA is self-replicating.' Discuss whether DNA needs anything else in order to replicate itself.

17 DNA polymerase synthesises DNA in a 5' to 3' direction. Does this mean DNA polymerase moves in the same direction as helicase on the leading and lagging strands?

Creating

18 Create a poster describing DNA structure.

19 Make a clear set of instructions for a cell to make a protein.

Reflecting

20 Watson and Crick used the contributions of scientists before them as a basis for their hypothetical model of DNA. Explain how your own understanding of DNA structure developed through your accumulation of knowledge.

PRACTICE EXAM QUESTIONS

1 In a DNA molecule, cytosine pairs with:
- **A** adenine
- **B** guanine
- **C** thymine
- **D** uracil.

[Q1 2018 SCSA]

2 If 30% of the bases in a DNA molecule are adenine, what percentage will be guanine?
- **A** 20
- **B** 30
- **C** 60
- **D** 70

[Q2 2018 SCSA]

3 In protein synthesis, transcription is the process whereby:
- **A** information in DNA is copied into mRNA
- **B** information in DNA is copied into tRNA
- **C** information in RNA is copied into tRNA
- **D** information in transfer RNA is copied into mRNA.

[Q10 2018 SCSA]

4 During DNA replication, the two new DNA strands are synthesised from the template strands at the same time. These strands are synthesised:
- **A** in the same direction
- **B** in opposite directions
- **C** by RNA polymerase
- **D** by DNA helicase.

[Q18 2018 SCSA]

5 DNA is made of units called nucleotides. Draw and label a diagram of a nucleotide. (5 marks)

[Q31a 2017 SCSA]

6 **a** List the two sets of complementary base pairs that occur in DNA molecules. (2 marks)

b Name the type of chemical bond that links the complementary base pairs in a DNA molecule. (1 mark)

c Name the base in mRNA that is complementary to thymine in DNA. (1 mark)

[Q31b (i, ii, iii) 2017 SCSA]

7 Describe the structure of mRNA (3 marks)

[Q31c 2017 SCSA]

8 Describe the role of tRNA in protein synthesis. (4 marks)

[Q31d 2017 SCSA]

9 Describe two differences between DNA and RNA molecules. (4 marks)

[Q31b 2016 SCSA]

10 Describe the structure of DNA and the main steps in DNA replication in a cell. (10 marks)

[Q36a 2016 SCSA]

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4 VARIATION AND MUTATION

CHAPTER 4 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 After a protein is made from a gene, how does this relate to an observable trait?
2 Can you explain how genes and the environment interact? Is there evidence for this?
3 Mutations can cause great harm in organisms, even death. How can they possibly be beneficial?
4 Are mutations the only source of variation in a population?

SCIENCE UNDERSTANDING

» the phenotypic expression of genes depends on the interaction of genes and the environment
» mutations in genes and chromosomes can result from errors in DNA replication or cell division, or from damage by physical or chemical factors in the environment
» variations in the genotype of offspring arise as a result of the processes of meiosis, including crossing over and random assortment of chromosomes, and fertilisation, as well as a result of mutations

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4.1 THE PHENOTYPIC EXPRESSION OF GENES

A **gene** carries a set of instructions for how to make a protein. When the gene is read, transcribed and translated into a protein, the gene is said to be **expressed**. The protein will have a specific function, depending on the gene. The protein may be a structural protein, contributing to the physical properties of cells or organisms. Examples are found in microtubules, muscle, membranes and hair. Alternatively, the protein may be an enzyme that catalyses one of the chemical reactions of cells. By coding for proteins, genes determine important facets of biological structure and function. These observable traits are known as the **phenotype**. However, genes cannot dictate the structure of an organism by themselves. The other crucial component involved is the environment. Twins may have the same gene and protein for a trait, but the trait may look different in the two individuals because the environment can influence the final phenotype. **Heredity** is the transmission of traits from one generation to the next. Although traits can be passed on, offspring are not identical to their parents, particularly in sexually reproducing organisms. There is a diversity of genetic and phenotypic traits within and between populations known as **variation**.

It is important to note that, within a multicellular organism, each of its cells has the same DNA and therefore the same genes. However, multicellular organisms have differentiated cells that perform specialised functions. Differentiated cells only have a small number of genes activated; for example, muscle cells only have those genes turned on that control muscle factors.

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FIGURE 4.1 Genetic variation can be observed in the phenotype of little penguins found on Penguin Island, Western Australia.

SCIENTIFIC LITERACY

A colourful example of variation: the Gouldian finch

A bird with vivid splashes of yellow, lilac and green hops onto a branch and tips her black-topped head from side to side. She is a Gouldian finch (*Erythrura gouldiae*). The Gouldian finch is a native inhabitant of the tropical grasslands of northern Australia, as well as a popular aviary bird. There are three distinctive forms distinguishable by head colour: red, black, and yellow-orange (Figure 4.2). They were once found across northern Australia in their millions, but now are distributed sparsely in small flocks in the Kimberley and Northern Territory.

The colour variation is associated with a suite of other differences in the birds. For example, the red-headed birds tend to be the most aggressive and frequently establish themselves at the top of the pecking order. By contrast, black-headed birds tend to be more inquisitive and are more likely to explore novel features in their environment. There are physiological differences, too. The red-headed birds are comparatively sensitive to starvation if food becomes scarce, and during breeding they respond by reducing the number of eggs they lay. Under the same conditions, however, black-headed birds continue to lay the same number of eggs and work harder to find food.

Females primarily seek a mate whose head colour matches their own. The head colouration is a mark of genetic compatibility. If a black-headed female mates with a red-headed male, comparatively few of their hatchlings survive

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FIGURE 4.2 The three forms of Gouldian finch

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to maturity. Around 60% of the sons and less than 20% of the daughters from such a pairing survive.

The Gouldian finch offers insights into the nature of variation. Variations in many features are observed between members of the same **species**. This is termed **intraspecific variation**. The form that any particular feature takes in an individual organism is described as a phenotype. Phenotypic variations can be classified according to whether they relate to the organism's appearance, chemical make-up or function.

What is the cause of the variation observed between individual birds? In essentially every case, the phenotype is shaped by the presence or absence of specific proteins and the activity of those proteins. For example, whether a bird's head feathers are yellow, black or red depends on the presence of specific enzymes that generate the pigments that colour the feathers. A bird's response to starvation is dependent upon the types and activities of the hormones and metabolic enzymes it has to sustain it during the period of an altered diet. As proteins are the products of genes, it is a straightforward conclusion that each phenotype has an underlying genetic basis.

Recall that **diploid (*2n*)** cells have two copies of every gene, each copy residing on one of a pair of **homologous chromosomes**. However, each copy of a particular gene is not necessarily identical. There are often small differences in the DNA sequence of the gene from one copy to another. These different versions of the same gene are called **alleles**. Essentially, all of the body cells of an individual Gouldian finch carry the same chromosomes. Each cell therefore possesses two alleles for any particular gene, and the two alleles may be the same or they may be different. If a single gene determines the colour of the head feathers, it is the combination of alleles the bird has (the **genotype**) that determines whether that colour will be yellow or black or red (the phenotype).

The colour of the head feathers, the position occupied in the social hierarchy, the response to environmental challenges, mate selection and many other features are, largely, an outward expression of the alleles each bird possesses. The Gouldian finch also demonstrates, however, that variation is not entirely explained by the alleles each individual has. The size a bird grows to and the physiological state of the animal are influenced by the availability of food. Breeding **behaviour** and outcomes are influenced by the availability of potential mates. To a greater or lesser extent, the organism's environment also plays a part.

Questions

1. State the colour variation you can observe in the finches in Figure 4.2.
2. Describe the main genetic source of the observed colour variation.
3. The literature describes other phenotypic variants, such as size, that are affected by the environment. Choose one and describe how the environment influences the phenotype.

Variation

Variation can be observed among siblings in a family, within a population of a species and between populations of a species. In this chapter, we explore the mechanisms that drive variation. Three main mechanisms will be discussed: environmental factors, **mutation** and sexual reproduction processes. Environmental factors may influence genotypic and phenotypic diversity. Mutation and sexual reproduction processes may increase genetic diversity. Genetic factors such as dominance, recessiveness, codominance and other allele systems also influence phenotype, and these will be discussed in Chapter 5.

A phenotype is an observable trait produced by the actions of one or more gene-encoded proteins. The phenotype is influenced by the genotype and the effects of the environment. The genotype is the genetic composition of an organism for a particular trait. It is the set of alleles that an organism has for a particular trait. An allele is a form of a gene. Different alleles code for the same trait but result in different versions of the trait.

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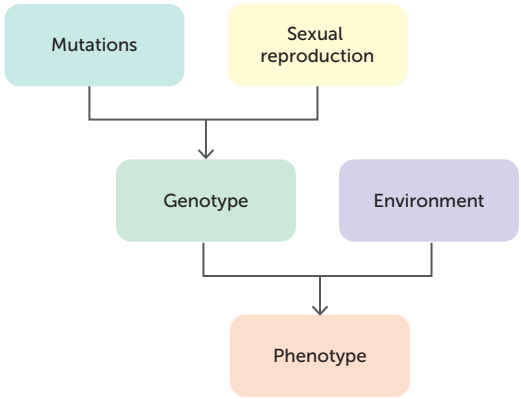

FIGURE 4.3 Phenotype is influenced by three mechanisms: mutations, sexual reproduction and environment.

Variation also exists between different species; for example, in penguins. Since penguins live at varying latitudes, and feathers account for nearly 85% of a bird's insulation, it should follow that different species would have different feathering patterns. All penguins maintain a body temperature of around 38°C, but they live in temperatures that range from 32°C on Penguin Island to −60°C on the sea ice of Antarctica. Banded penguins, such as Humboldt and African penguins, have featherless patches on their faces and feet to which they divert blood for cooling when they are overheating. In contrast, the Adélie penguin, one of two Antarctic species, has complete feather coverage up to the base of its beak.

Key concept

The interaction between genes and the environment leads to variation in observable traits (the phenotype) of individuals.

Question set 4.1

REMEMBERING

1 Define:
   - **a** variation
   - **b** phenotype
   - **c** genotype
   - **d** allele.

2 Name the main sources of genotypic variation.

3 State two mechanisms of phenotypic variation.

UNDERSTANDING

4 Explain the relationship between the terms alleles, genotypes, proteins and phenotypes.

APPLYING

5 Penguins are found in various environments, with temperatures ranging from −60°C to 32°C. Calculate:
   - **a** the mean temperature
   - **b** the median temperature.

4.2 ENVIRONMENTAL FACTORS

Phenotype is shaped by the presence or absence of specific proteins and the activity of those proteins. In turn, phenotypic expression of genes depends on the interaction of genes and environmental factors. The alleles for a particular gene are found on homologous chromosomes and form the genotype. The genotype strongly influences the phenotype, but there can be a range of phenotypes due to environmental influences. Genotypes for height, size, skin colour and flower colour are some traits that can have a range of phenotypes given the same genotype. However, there are some traits that are strictly defined by genotype, such as the ABO blood group system.

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Environmental factors that influence phenotype can be external or internal. External environmental factors include temperature, pH, availability of food, light exposure and wind exposure. For example, for crocodiles, alligators, and certain lizard and turtle species, an embryo can become either a male or a female depending on the temperatures it experiences while in the egg.

Another example of phenotype being affected by the external environment is hydrangea flower colour. The range of colours can be traced back to the pH in the soil, and ratios of additives such as aluminium ions. A more acidic soil is conducive to blue flowers. The pH does not change the **genome** (the total DNA content of the individual organism) because the genotype does not change. It is the interaction of the environment with either the gene or the protein it codes for that determines the sex in turtles and the flower colour in hydrangeas.

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FIGURE 4.4 Soil pH can affect flower colour in hydrangeas.

Rising temperatures turning sea turtles female

CASE STUDY

Researchers studying green sea turtles found rising global temperatures were affecting female-to-male ratios faster than expected. The ratio of males to females was in decline. Sea turtles reproduce sexually, so the reduction in numbers of males puts sea turtles at risk of extinction. The sex of a sea turtle is a phenotype that is affected by an environmental factor – temperature. Sea turtles bury their newly laid eggs in sand. The temperature of the sand determines the sex phenotype. Cooler temperatures produce males; warmer temperatures, 29.1°C and above, produce females. Relatively recently, rising air and water temperatures have affected the amount of heat gained by the incubating sand. Consequently, scientists predicted a slight increase in numbers of females and a decrease in numbers of males. Scientists found they were underestimating the rate of change in the ratio. A study on a sea turtle rookery in the Pacific Ocean found the ratio of females to males was 116:1.

Marine biologist Michael Jensen teamed up with other experts to test Great Barrier Reef sea turtles' sex to find out if climate change had already altered the ratio of males to females in hatchlings. Scientists had to use invasive techniques to find out the sex of individual turtles. Blood samples were taken to test for specific hormone levels. On Raine Island (off the coast of northern Queensland), most of the results showed females. This is an extreme change, considering that in the 1970s a study showed along the northern Great Barrier Reef that the ratio of females to males was around 6:1.

Temperatures continue to rise, and so the temperatures of sand where eggs are incubating continue to be affected. What has scientists most concerned is the rate at which the temperatures are changing and the relatively little time animals have to adapt to the change. Average global temperatures are predicted to increase 2.6°C by 2100. Scientists are investigating ways to help reduce the impact of rising temperatures on the eggs. Strategies include the creation of natural and artificial shade over areas where eggs are laid, and watering the sand to cool it down. These strategies have resulted in a healthier ratio of male to female hatchlings. Many scientists are hopeful that turtle species can eventually become independent again by adapting to the changes.

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FIGURE 4.5 Temperature affects sex determination in sea turtles.

Questions

1. Explain the cause of the abnormal male-to-female ratios in the green sea turtles?
2. Describe the impact this may have on green sea turtles over the long term.
3. Evaluate the method used in this case study to re-establish a more natural sex ratio. Can you design a better method?

Internal environmental factors include the action of hormones. For example, the release of gonadotrophin-releasing hormone (GnRH) triggers the start of puberty in humans. Some hormones are driving forces for growth, and low levels can result in small birth weights and slow development. A group of veterinary drugs called 'hormonal growth promotants' (HGPs) mimic cattle growth hormones and are used in Australia to increase muscle growth and meat yield.

Epigenetics can affect phenotype

Epigenetics research
Be inspired by the sketch video. Try to make your own sketch.

Lick your rats
Can the epigenome be inherited even though it does not change the genome? And are epigenetic changes reversible?

The study of inheritable (but reversible) changes in gene expression without a change in the DNA sequence is called **epigenetics**. The prefix 'epi' means above. In relation to the genome, the epigenetic factors are 'above' the DNA and exert control over it by activating and deactivating genes without altering the DNA. Epigenetics can influence phenotype by controlling gene expression.

Environmental factors can contribute to the addition or subtraction of epigenetic chemical factors and turn certain genes on or off. Environmental factors such as diet and stress can also affect **chromatin** structure and gene expression. For example, the addition of an acetyl group to the chromatin structure appears to promote transcription, and turn a gene on. However, the addition of a methyl group to a histone can reduce transcription, and turn a gene off. Removal of some methyl groups can also turn genes on. Chromatin modifications via histone acetylation and DNA methylation do not change the DNA sequence, yet they may still be passed on to future generations.

Epigenetics affects gene expression, and therefore it can affect the growth and development of organisms. A mother who smokes during pregnancy can cause epigenetic factors to be modified in her unborn child, causing the child to be more at risk of obesity. When the epigenome is altered, it does not alter the DNA sequence permanently and is therefore not a mutation. However, changes to the epigenome can still affect the phenotype.

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Key concept

Internal and external environmental factors can influence phenotype. Internal environmental factors include hormones, which are chemical messengers. External environmental factors include temperature and access to nutrients. Epigenetics can also influence phenotype by controlling gene expression.

Question set 4.2

REMEMBERING

1 List four environmental factors that can affect the phenotype in a specified organism, and classify them as internal, external or epigenetic.
2 Name four phenotypes in mammals that display variation due to the environment.
3 Explain how epigenetics can affect phenotype. Use an example to demonstrate your explanation.

UNDERSTANDING

4 Using a real example, describe how the interaction of a gene and the environment can affect the phenotypic expression of a gene.

4.3 MUTATIONS CAUSE VARIATION

Permanent changes to an organism's DNA sequence are termed mutations. Mutations may arise spontaneously during DNA replication or cell division (**spontaneous mutations**), or they may be induced by physical or chemical environmental factors called **mutagens**, or through the action of biological agents. Mutations that occur in genes often affect the proteins they code for. These effects are sometimes subtle. More often they are severe, with potentially catastrophic effects for the survival of the organism that bears them. Rarely, they can enhance the function of the protein or make an organism better suited to the environment it inhabits. The effect of a mutation depends upon whether it has occurred in non-reproductive (body, or **somatic**) cells or in reproductive (**germline**) cells.

A mutation in a somatic cell only affects the body cell in which it occurs and the daughter cells produced from it by mitosis (Figure 4.6). All other cells of that organism lack the mutation. Cancer is a consequence of some mutations in somatic cells. Mutations associated with cancer can occur in particular genes or regions of the DNA, and they accelerate the rate of cell division, affect the cell's ability to undergo **apoptosis** or increase the rate of mutations within the cell.

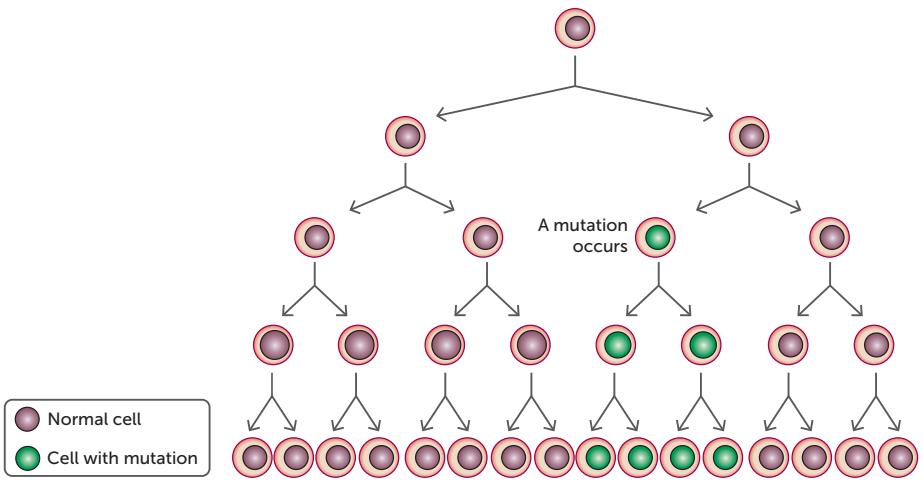

FIGURE 4.6 A mutation in a somatic cell affects only the cell in which it occurs and its daughter cells.

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Mutations that occur in germline cells affect sex cells called **gametes**, and they have the potential to be inherited (passed on to the next generation) and be incorporated into every cell of the offspring (Figure 4.7). Often, the germline mutation results in developmental abnormalities that cause the affected embryo or foetus to be spontaneously aborted. If carried through to birth, the germline mutation may result in congenital disorders in the offspring, with varying degrees of severity. Occasionally, a gene mutation changes or enhances the function of the protein that it codes for. If circumstances suit, it can enhance the survival of the organism. If the mutation is consistently passed on from one generation to the next, a new allele becomes established in the population. Such mutations in germline cells may contribute to the species' gene pool and can influence whole populations and their evolution.

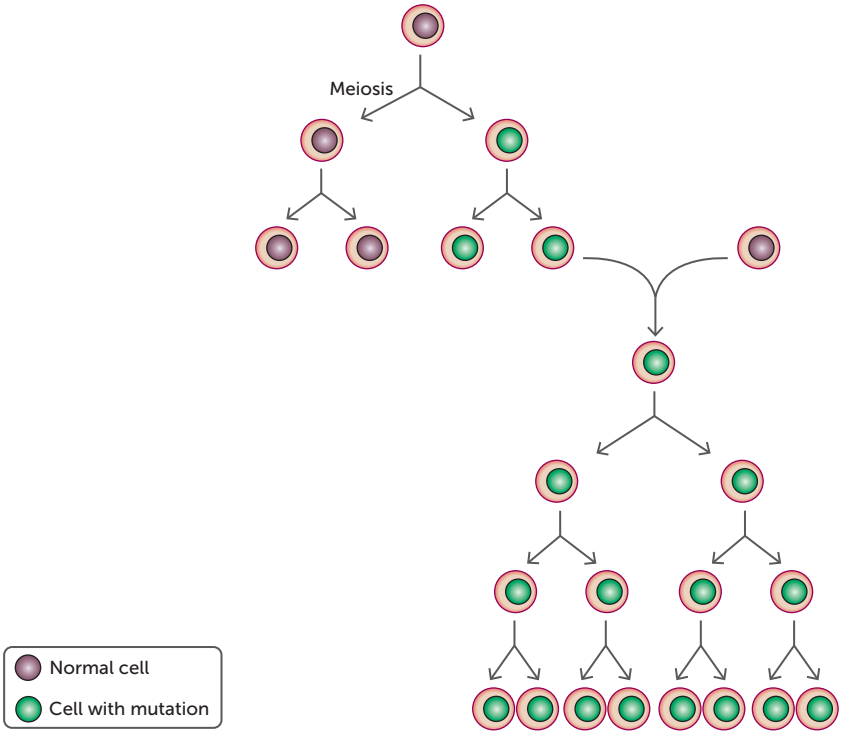

FIGURE 4.7 Mutations in germline cells affect all body cells of the individual organism that inherits them.

Recessive mutations that lead to a loss of function can be masked if a normal copy of the gene is present; for the **mutant** phenotype to occur, both recessive alleles must contain the mutation. Dominant mutations lead to a mutant phenotype even in the presence of a normal copy of the gene. The phenotypes associated with dominant mutations may represent either a loss or a gain of function.

Question set 4.3

REMEMBERING

1 Define:
- **a** mutation
- **b** mutagen
- **c** somatic cell
- **d** germline cell.

2 Describe two main differences between when a mutation produces a dominant allele and when a mutation produces a recessive allele.

UNDERSTANDING

3 Differentiate between mutations that occur in somatic cells and mutations that occur in germline cells.

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4.4 CAUSES OF MUTATIONS: ERRORS IN DNA REPLICATION AND CELL DIVISION

DNA replication errors

Spontaneous mutations can occur during the S (synthesis) phase of the cell cycle, when the DNA is exposed during replication and is vulnerable to damage. DNA replication is an extremely accurate process, but mistakes can occasionally occur when DNA polymerase inserts the wrong nucleotide. Adenine, for example, normally base-pairs with thymine, but may spontaneously undergo a chemical change that makes it resemble a guanine, which pairs with cytosine. During DNA replication, the chemically different form of adenine may be mistaken, and a nucleotide containing guanine may be introduced into the DNA sequence instead. Repair mechanisms can correct the mistakes, but in rare cases mistakes are not corrected, leading to mutations.

Errors may also be introduced into DNA sequences by highly corrosive chemicals containing oxygen. These chemicals, termed reactive oxygen species, may be generated naturally by the cell's own metabolism or by the action of mutagens. Enzymes in the cell, such as catalase, remove many of these chemicals, but if there is for any reason an excess of reactive oxygen species, they readily react with DNA to cause damage to the DNA structure.

During the G2 phase of the cell cycle (see Chapter 2, pages 31–32), DNA is proofread, and any errors that are detected are repaired. Repair often depends on one of the DNA strands being intact. The intact strand serves as a template for proofreading and restoration of the damaged complementary strand. However, if a mutation is not repaired or it is repaired improperly, the mutation becomes part of the DNA sequence and persists through subsequent cell divisions (Figure 4.8).

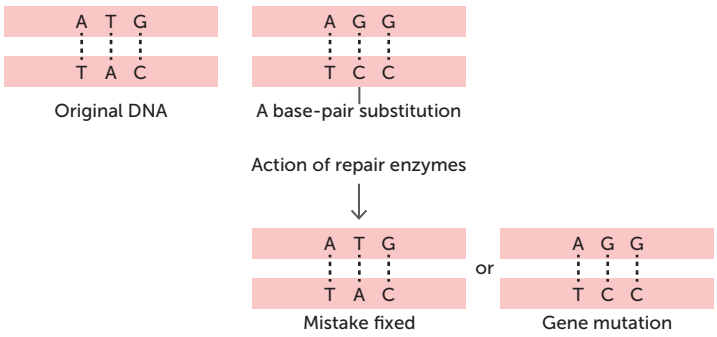

FIGURE 4.8 Base-pair substitution results in either a mistake being fixed or a mutation.

Mutation studies

The DNA repair mechanisms are usually highly effective, so mutations are comparatively rare. **Mutation rates** vary, however, from one species to another. Low mutation rates made it difficult for geneticists to investigate mutations until the discovery in 1927 by an American biologist, H.J. Muller, that the mutation rate in the fruit fly (*Drosophila melanogaster*) can be greatly accelerated by irradiation with X-rays. Since then, it has been found that other environmental mutagens speed up the mutation rate. The discovery of mutagens made it easier to study the cause and transmission of mutations. Bacteria and plants are used in most experiments, although scientists also perform experiments on animal cells using tissue culture techniques.

From these studies, three main ideas have emerged.

First, mutations arise spontaneously and are not directed by the environment. Environmental influences can greatly affect the mutation rate, but they cannot induce a particular mutation to occur.

Second, mutations are persistent. They tend to be transmitted through many cell divisions without further change, although there is always the possibility that another mutation may occur, either producing a new feature or a return to the original condition.

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Third, the majority of mutations confer disadvantages on the organisms that inherit them. The premature death of organisms with harmful mutations (before reproductive age) prevents harmful mutations accumulating in populations. The occurrence of a useful mutation is an extremely rare event.

Cell division errors

Mutations can occur in somatic (body) cells during mitosis, or in the germline cells in the gonads during **meiosis**, when gametes are formed.

Mutations can result from a number of events, including unequal **crossing over** during meiosis (Figure 4.9). If non-sister chromatids misalign during crossing over, one gamete may gain extra nucleotides (leading to an **insertion mutation**), and one may lose some nucleotides (leading to a **deletion mutation**). (Crossing over does not happen during mitosis.)

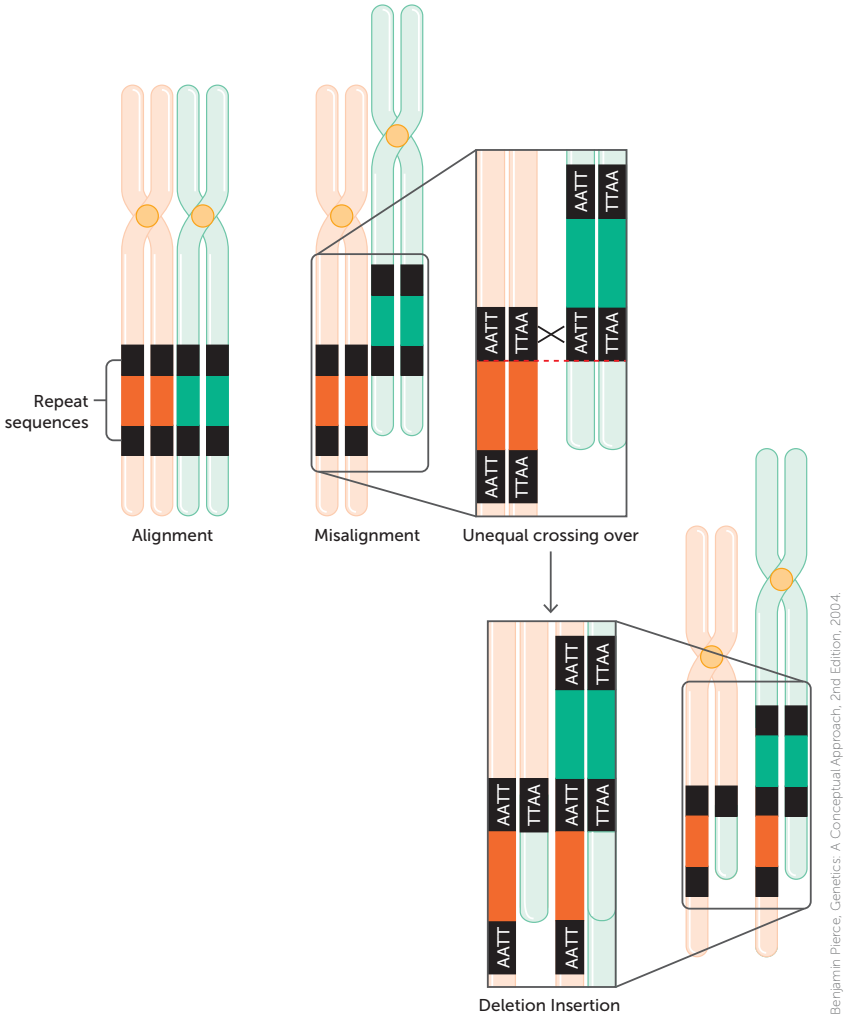

FIGURE 4.9 When homologous chromosomes misalign during meiosis, unequal crossing over may occur. The result is the deletion of a DNA sequence in one chromosome, and the insertion of a DNA sequence in the other chromosome.

Larger-scale mutations can occur during anaphase in mitosis and during anaphase I or anaphase II in meiosis when homologous chromosomes or sister chromatids do not separate at the centromere. These larger chromosomal mutations will be discussed later in the chapter.

Key concept

Mutations in DNA can cause permanent changes in genes and chromosomes, and can lead to variation within species. Mutations can arise spontaneously during DNA replication. Insertion and deletion mutations can arise during crossing over during meiosis.

Question set 4.4

REMEMBERING

1 Recall the purpose of DNA replication.
2 Describe the ways in which a mutation can occur during DNA replication.
3 Relate how the discovery of mutagens helped scientists understand mutations.

UNDERSTANDING

4 Explain how a mutation can occur during crossing over in anaphase I. How would this affect gamete formation?
5 If a spontaneous mutation occurs during DNA replication, will it always be passed on during cell division?

4.5 CAUSES OF MUTATIONS: MUTAGENS

Physical mutagens

Physical mutagens include various types of high-energy radiation that cause DNA damage (Table 4.1). One example is ultraviolet light (UV light), a natural component of sunlight. Public awareness campaigns have drawn attention to the risks of excessive exposure to UV light, such as increased risk of skin cancer. Physical mutagens often affect the nitrogenous bases of DNA, causing distortions in the double helix. UV light, for example, fuses adjacent thymines or cytosines in the DNA sequence. Ionising radiation, such as X-ray irradiation, causes the loss of adenine and guanine bases, although the DNA backbone remains intact, creating gaps in the double helix. These aberrations disrupt complementary base pairing. Ultimately, incorrect bases may be inserted in their place during DNA replication.

TABLE 4.1 Some physical mutagens and their effects

PHYSICAL MUTAGEN	EFFECT
UV light	Structural distortion by cross-linking neighbouring nucleotides
X-rays	Gene and chromosome aberrations
Nuclear radiation	Breaks in DNA strands

Physical mutagens frequently also cause **double-strand breaks**, which are essentially complete breaks in the chromosomes (Figure 4.10). Sometimes the broken ends leave single-stranded overhangs that are complementary to one another. This enables them to bind to one another and facilitates repair of the broken sequences. Other times the double-strand break has no overhangs, or the DNA at the fragment ends is damaged so that these ends no longer match. In such cases, mistakes can occur during repair, and the consequences may be especially hazardous to the cell. Broken ends can be rejoined inappropriately to the wrong fragments of DNA. Intervening segments of broken DNA can be lost. These kinds of anomalies result in chromosomal rearrangements. An accumulation of double-strand breaks that occurs upon intense exposure to physical mutagens is often lethal to the cell. Apoptosis of the cell in this situation helps to guard against cancer formation.

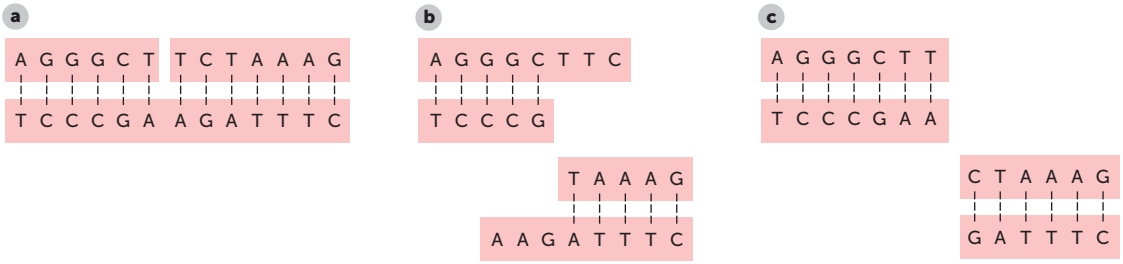

FIGURE 4.10 Compare **a** a single-stranded break in DNA with **b** and **c** double-stranded breaks. The double-stranded break in **c** is the most difficult of the three for the cell to repair.

Chemical mutagens

The mechanisms by which chemical mutagens exert their effects vary (Table 4.2); however, a common outcome is the substitution of one nucleotide for another. Mustard gas was introduced in World War I as a chemical warfare agent, and was also found to be mutagenic during World War II experiments. Sulfur mustard is a powerful irritant and blistering agent that damages the skin, eyes and respiratory (breathing) tract. Sulfur mustard damages DNA, especially in the bone marrow. It can be absorbed through the skin or inhaled.

Some chemical mutagens, such as 5-bromouracil, act directly as a substituting base. The 5-bromouracil resembles thymine and can become incorporated in place of it during replication. However, unlike thymine, the incorporated 5-bromouracil can form hydrogen bonds with either adenine or guanine. The ambiguous pairing affects DNA replication during subsequent cell divisions, leading to a C–G pair being swapped for the original T–A pair.

TABLE 4.2 Some chemical mutagens and their effects

CHEMICAL MUTAGEN	EFFECT
Mustard gas (sulfur mustard)	Mustard gas affects the base guanine, causing a substitution mutation
2-aminopurine, 5-bromouracil	Nucleotide substitution
Colchicine	Prevents spindle formation in mitosis and so doubles chromosome number
Nitric acid	Adenine in DNA is deaminated so it behaves like guanine

Biological agents

Genetic mutations sometimes arise because of the action of invasive pathogens, such as bacteria and viruses. Occasionally, the DNA of these pathogens becomes permanently integrated into the host cell's DNA, causing mutations in subsequent daughter cells (as in the case of Tasmanian devil facial tumour disease, see page 45).

Bacteria and viruses, and horizontal gene transfer

Bacteria of the genus *Agrobacterium* cause crown gall disease in the stems of plants of several species (Figure 4.11). The bacterium achieves this by inserting a **plasmid**, called a Ti plasmid, into a cell of the host plant. The Ti plasmid contains genes that code for enzymes that cut the host plant's DNA and integrate a segment of the Ti plasmid into it. The cell of the host plant thus becomes modified by **horizontal gene transfer**. The integrated bacterial DNA contains additional genes that essentially hijack the host plant cell machinery to produce nitrogen- and carbon-rich compounds that the bacterium uses as a nutritional source. The infected cell is also induced to produce hormones that stimulate the plant cells to rapidly divide and grow. The increased rate of cell division results in the formation of the distinctive tumour-like gall that is, in effect, a food factory that sustains the expanding population of bacteria. The capacity to carry out horizontal gene transfer has made specially engineered strains of *Agrobacterium* a valuable **cloning vector** for genetically modifying plants.

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A number of viruses are also capable of horizontal gene transfer. Notable among them is the human papillomavirus (HPV), which infects epithelial cells of human skin and mucosal membranes.

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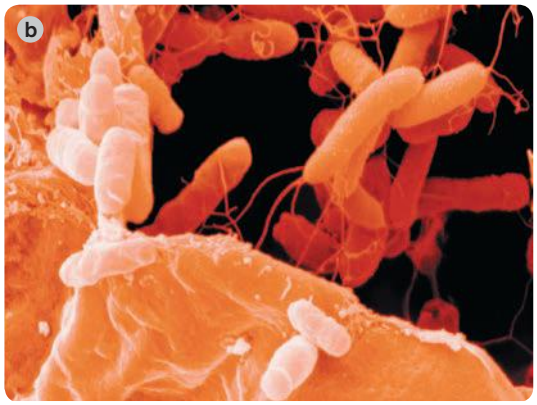

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FIGURE 4.11 a Crown gall disease of a rose bush caused by **b** the bacterium *Agrobacterium tumefaciens*

Key concept

Environmental factors that cause mutations are called mutagens. Mutagens can be physical, chemical or biological.

Question set 4.5

REMEMBERING

1 Complete the following table:

MUTAGEN	EXAMPLE	EFFECT
Physical		
Chemical		
Biological		

2 Define double-strand break.

3 Explain the difference between a physical and a chemical mutagen.

UNDERSTANDING

4 Explain how bacteria of the genus *Agrobacterium* causes crown gall disease in plants.

APPLYING

5 A unique segment of DNA consisting of 2907 nucleotide pairs first appeared in the genome of the wild fruit fly (*Drosophila melanogaster*) in the mid-20th century. Since then, it has spread throughout wild populations and increased in copy number within individual flies. Discuss what might account for these changes in the fruit fly DNA over the last half century.

4.6 TYPES OF MUTATIONS

Point mutations

The simplest form of mutation is a **point mutation**, in which just a single nucleotide within the original DNA sequence is affected by a substitution, addition or deletion.

Substitution

A substitution occurs when one nucleotide is replaced by another (e.g. adenine substituted for guanine). **Substitution mutations** are a source of novel SNPs and have a number of possible effects on the translated protein.

Point mutation simulation
Use the simulator to perform transcription and translation, then edit the DNA to observe the effects of a point mutation.

Mutations activity
Find out how a point mutation can alter a gene.

Differences between sequences of just one nucelotide are also called **single nucleotide polymorphisms** (SNPs, often pronounced as 'snips'). If the SNP occurs in a gene, the mutated gene sequence can be transcribed and translated into a protein that is the same as that encoded by the original form of the gene, or it may be altered. When the protein is altered, the mutation may have a subtle or a dramatic effect on its structure and function.

A **synonymous mutation**, also referred to as a **silent mutation**, occurs when the substituted base results in a **codon** (also known as a triplet) that codes for the same amino acid as the original codon. For example, AGA and AGG both specify the addition of an arginine amino acid in a polypeptide chain (Figure 4.12). The protein encoded by the mutated gene is therefore identical to that encoded by the original gene. Synonymous mutations are possible because there is a level of redundancy in the **genetic code**. Recall that the genetic code consists of 64 codons that code for 20 amino acids and the instructions to start and stop translation. Therefore, any individual amino acid can be encoded by more than one codon.

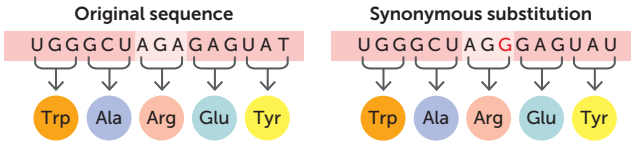

FIGURE 4.12 Synonymous mutation

A **missense mutation** arises when a SNP changes the amino acid. For example, substitution in an AGA codon to generate an AGC codon results in a serine amino acid being added to the polypeptide instead of the original arginine (Figure 4.13).

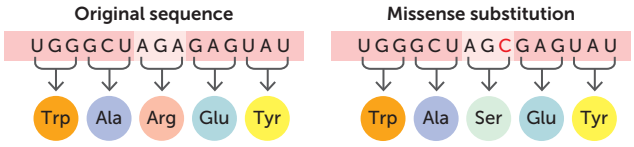

FIGURE 4.13 Missense mutation

A **nonsense mutation** occurs when a SNP creates a new stop codon within the original gene sequence (Figure 4.14). This leads to early termination of translation of the transcribed gene sequence. As the remaining sequence downstream of the new stop codon is not translated, the result is the production of an incomplete polypeptide.

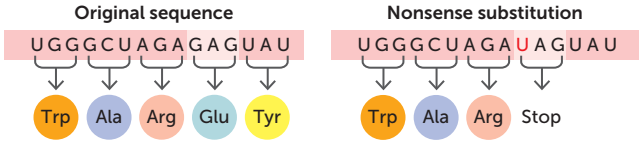

FIGURE 4.14 Nonsense mutation

Insertions and deletions

As the name suggests, an insertion mutation is the addition of one or more nucleotides at a site within the original gene sequence. A deletion mutation is the loss of nucleotides from a site within the original gene. The effect of this type of mutation is frequently a **frameshift mutation**, in which the reading frame for the corresponding amino acids has been nudged away from the original, and all the codons downstream of the mutation are affected. The consequence for the translated protein is that the amino acids downstream of the mutation bear no resemblance to those of the original polypeptide (Figure 4.15). Under such circumstances, even a single nucleotide insertion or deletion can have a profound effect on the corresponding protein.

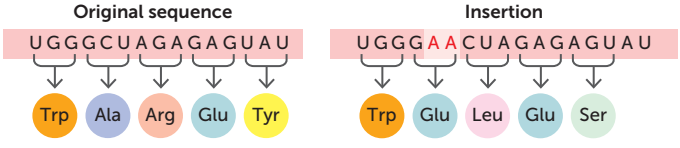

FIGURE 4.15 An insertion in the gene sequence results in a frameshift mutation.

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Key concept

Point mutations can cause changes in a DNA sequence by either (i) substitution of a nucleotide (SNP) or (ii) insertion or deletion of nucleotides (indels). Substitutions can be classified as synonymous, missense or nonsense mutations. Insertion and deletion mutations are classified as frameshift mutations.

Effects of mutations on survival

A protein's function is dependent on its structure. Mutations that change a protein's structure can have consequences for protein function, with potential impacts on the organism's survival. Mutations can therefore also be classified according to whether the effect of the mutation on the protein's function and the organism's survival is unchanged, changed for the worse, or changed for the better.

Neutral mutations

In the case of synonymous mutations, the protein product is unchanged compared with the original, so the organism's survival is unaffected by the change. This is said to be a **neutral mutation**. Missense substitutions are sometimes also neutral mutations, provided that the original amino acid is swapped with another that has similar properties. For example, in the *ABCA1* gene, which codes for a protein involved in cholesterol transport, a missense substitution in a single GAA codon creates a GAC codon and causes one amino acid (glutamic acid), to be swapped for another (aspartic acid). Both amino acids are negatively charged, however, and reside on the surface of the protein, where they interact with the surrounding water, so the properties and function of the protein remain essentially the same.

Deleterious mutations

A living organism can be compared with a complex product of engineering, such as an aeroplane, in which the components are so intricately integrated that an indiscriminate change to any component can harm the overall operation of the aircraft and make it unfit to fly. Similarly, random mutations may disrupt the function of the encoded protein, undermining the organism's overall ability to carry out its basic processes and survive. Such mutations are referred to as **deleterious mutations**. The majority of mutations are deleterious.

Nonsense mutations are typically deleterious, because they result in the production of an incomplete protein that is non-functional. However, these deleterious mutations may persist if the individual who carries them also has a copy of the normal allele that encodes the functional version of the protein. The deleterious mutation is thus masked within the phenotype of the organism. If the organism is unfortunate enough to have only non-functional alleles for a particular gene, the condition usually results in the death of the organism before it has the opportunity to reproduce and pass the alleles on to any offspring.

Beneficial mutations

Occasionally, gene mutations produce a new allele that benefits the survival of the organism. The type of **beneficial mutation** can vary: it could be a missense mutation that changes the function of the original protein, or it could be a nonsense mutation that eliminates a protein that may have been harmful to the organism in some circumstances.

The human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS). Without treatment, AIDS is fatal in essentially all cases. A few individuals have been exposed to the virus but have proved to be resistant to infection. These individuals have a nonsense mutation that results in the elimination of one of the surface proteins required by HIV to enter cells. This deletion confers resistance to HIV infection.

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Key concept

Mutations can be categorised as neutral, beneficial or deleterious, depending on the effect they have on the survival of the individual.

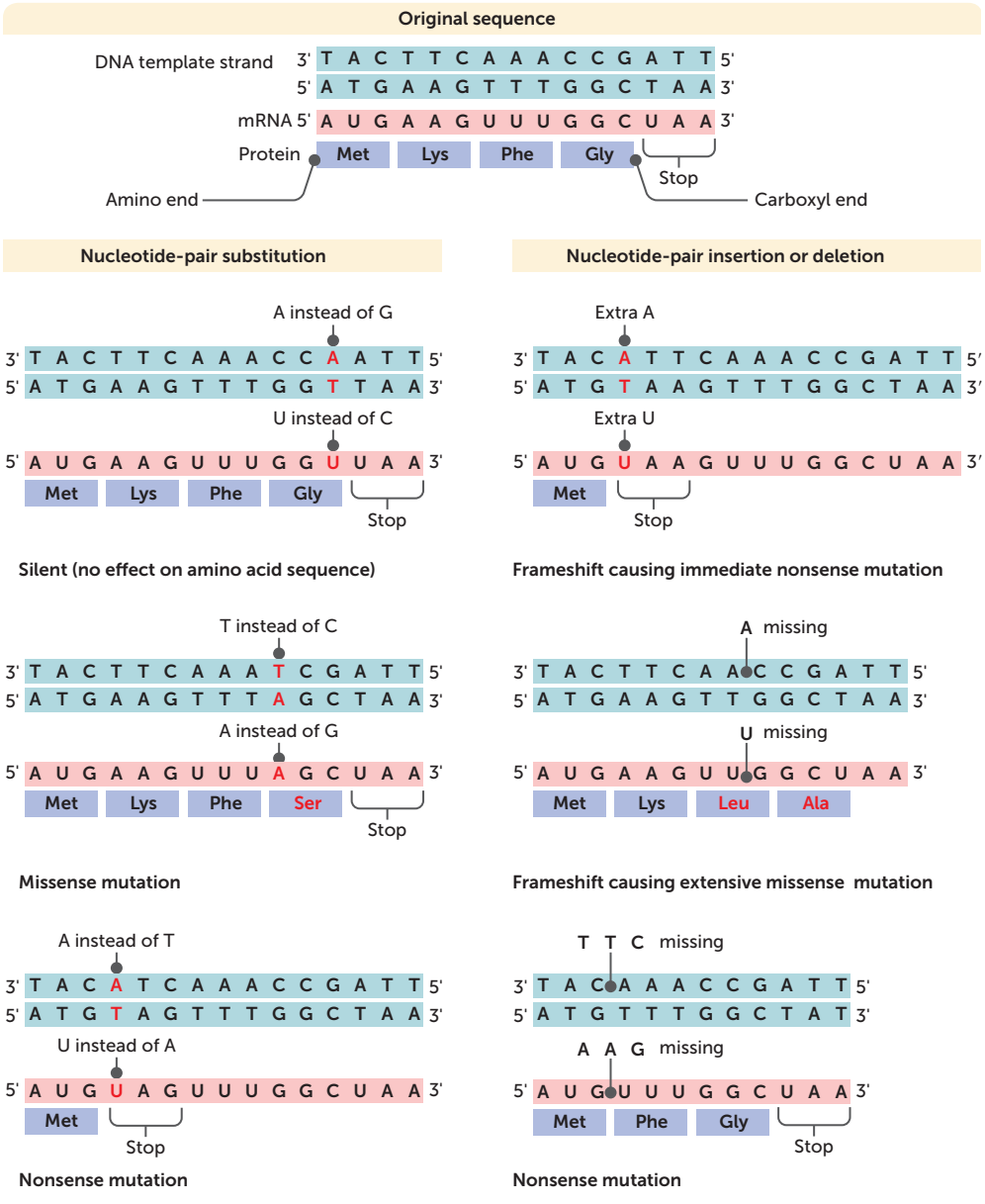

FIGURE 4.16 The effects of point mutations on transcription and translation

Question set 4.6a

REMEMBERING

1 Define point mutation.
2 Describe the following types of genetic mutations:
   a substitution
   b insertion
   c deletion.

3 Describe the effect of the following mutations on a coded protein:
- **a** synonymous mutation
- **b** missense mutation
- **c** nonsense mutation
- **d** frameshift mutation.

UNDERSTANDING

4 Classify the following mutations as neutral, deleterious or beneficial to an organism's chances of survival.
- **a** An indel in the human hexosaminidase A gene results in improper neural development.
- **b** A mutation in the beta-lactamase gene of the bacterium *Escherichia coli* generates a new version of the enzyme that detoxifies the antibiotic ampicillin.
- **c** A nonsense mutation in the human *SURF1* gene encodes a protein crucial for formation of a key metabolic enzyme.
- **d** A synonymous mutation in the codon for an amino acid occurs at the active site of bovine salivary amylase.
- **e** Various mutations in a gene for the enzyme alcohol dehydrogenase result in different versions of the functional enzyme.
- **f** A mutation that extends expression of a human lactase gene enables lactose digestion to continue into adulthood.

Chromosome mutations

Genetic variations can also occur because of wholesale changes to the chromosomes. Alterations to chromosomes differ from single point mutations because they can affect many genes simultaneously. Some of the variations that occur with chromosomes, such as chromosome number, are quite natural in certain situations and are therefore integral to the functioning and continuity of the species. Others arise because of anomalies that occur during the formation of the gametes.

Chromosome alterations can be observed and analysed by examination of a prepared microscope slide of stained cells photographed in the process of nuclear division. This reveals a jumbled cluster of chromosomes that differ in size, shape and banding. Photographic images of chromosomes can be rearranged into matched and ordered pairs to create a **karyotype**, the standard format used to display and analyse chromosomes (Figure 2.3, page 28). Chromosomes are ordered by length, from largest to smallest, and they have characteristic banding patterns. Species are characterised by having a particular number of chromosomes in each cell.

Variations in chromosome number

In many eukaryotic organisms, the somatic cells are diploid (*2n*): the cells contain two sets of chromosomes, with one set inherited from each parent. The gametes are **haploid (*n*)**. There are consequences for organisms when the complement of chromosomes in the somatic cells varies from the usual diploid state.

Monoploidy

In many colonial insects such as ants, bees and wasps, the males of the species are **monoploid (1*n*)** (Figure 4.18). By contrast, the females, including the queen, are diploid. The males do not

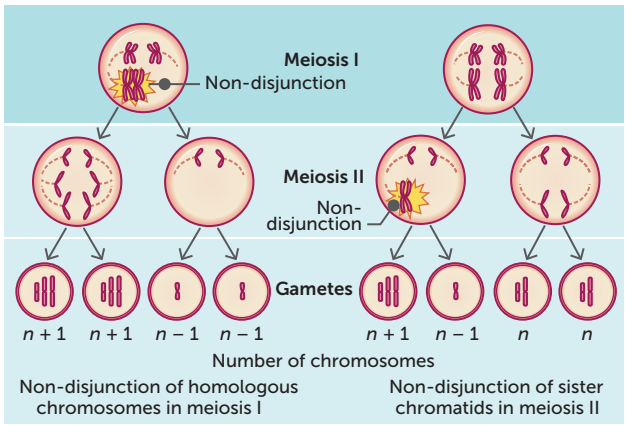

FIGURE 4.17 Chromosome non-disjunction and its effects on gametes

need to become diploid to function, as do the haploid gametes of regular diploid animals. Their chromosomes represent a single complete and operational set, and the males function like any other multicellular animal. In contrast, in haploid gametes, the chromosomes represent half of the complete set and are packaged in a dormant state, awaiting the **fertilisation** event that will activate them. In these insects, the queen produces eggs by meiosis, whereas the males produce sperm by mitosis. Fertilisation results in diploid female offspring. The males are instead produced by **parthenogenesis**, a process in which an entire organism is regenerated from a single egg cell, without the need for fertilisation.

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FIGURE 4.18 Bees maintain their colony structure with diploid females and monoploid males.

Many fungi and algae are also monoploid, and there are examples of monoploid fish, amphibians and reptiles. Monoploidy seems economical because only one set of chromosomes is required, so why are diploid organisms so much more common? The advantage for diploid organisms is that any defective alleles that arise can be compensated for by a functional allele on the corresponding chromosome. In monoploid organisms, a defective allele is the only allele available for a particular gene, and the consequences are likely to be deleterious.

Polyploidy

Sometimes the cell divisions that give rise to haploid gametes fail altogether, so that half the gametes contain two copies of each chromosome (diploid, 2*n*) and the rest have none. If a diploid gamete fuses with a normal haploid gamete, the resulting individual is triploid (3*n*): it has three of each type of chromosome. If two diploid gametes fuse, a tetraploid (4*n*) individual will be produced. It is therefore possible for an organism to acquire one or more complete extra sets of chromosomes, a phenomenon called **polyploidy**.

Polyploidy is particularly common in flowering plants, ferns and green algae; approximately half of all flowering plant species are polyploid. Polyploidy also occurs in fungi and in some fish and amphibian species. There are advantageous commercial applications of polyploidy in plants: for example, increased fruit size, hardiness and infertility, which lead to increased profits for farmers and businesses (Figure 4.19).

Polyploidy is lethal in humans. In the rare situation of a pregnancy continuing to a live birth (1% of human polyploids), the newborn dies within a month.

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FIGURE 4.19 The application of polyploidy to create infertile fruit, such as seedless grapes, is of considerable commercial significance.

Aneuploidy

Tasmanian devils and aneuploidy
Read about aneuploidy in Tasmanian devils.

Aneuploidy is the condition in which there is an addition or loss of one chromosome (or a few chromosomes) from a cell (i.e. $2n + 1$ or $2n - 1$). Tasmanian devil populations have suffered from a facial cancer that is infectious. The Tasmanian devil facial tumour cells have been karyotyped and compared with a normal devil's karyotype. Significant aneuploidy (one or more extra or missing chromosomes) was evident. Reproductive failure by miscarriage is common, and it has been found that many miscarried embryos are aneuploids. To understand how this comes about, consider the process of meiosis. Normally in meiosis, identical chromosomes come together and then segregate into separate cells, so that the gametes finish up with only one of each pair of chromosomes. Occasionally, however, the two identical chromosomes do not separate, but go into the same cell. This phenomenon is known as **non-disjunction**. Generally, non-disjunction only takes place with one pair of homologous chromosomes, while the rest behave normally. It can occur during either the first or second meiotic division. Non-disjunction results in the formation of two types of gametes in equal proportions, but one type has two copies of a particular chromosome and the other type has none (Figure 4.20).

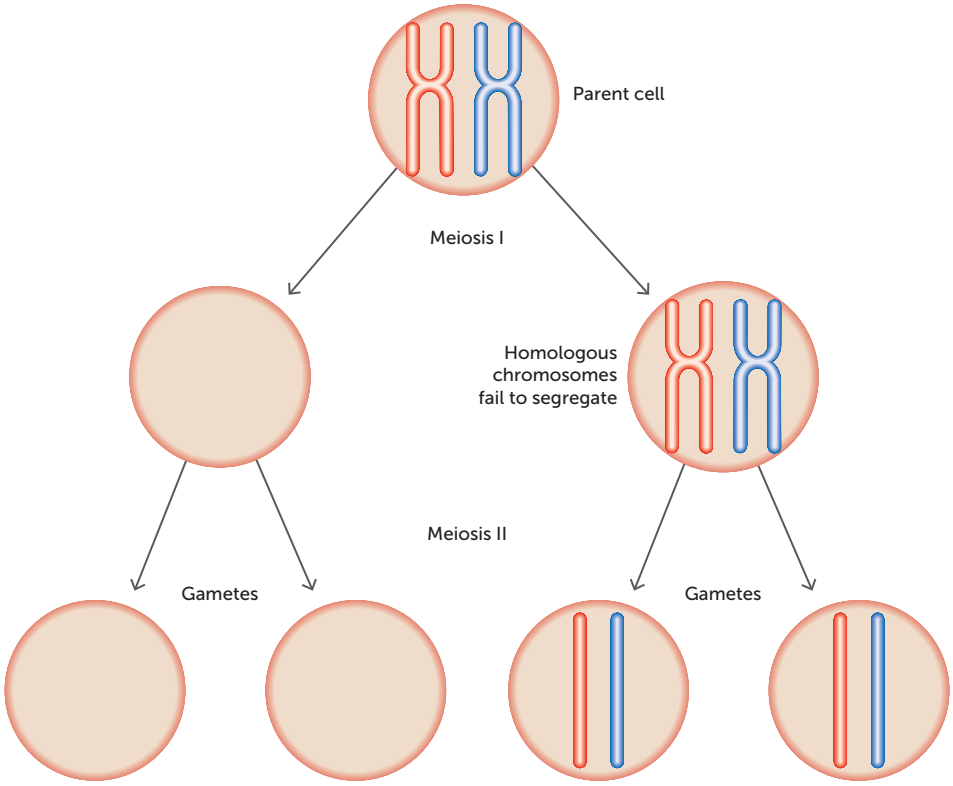

FIGURE 4.20 In non-disjunction, the chromosomes fail to segregate, so half the gametes contain two chromosomes of a pair (bivalent) each, and the other half contain no chromosomes at all.

The fusion of a gamete containing both homologous chromosomes with a normal gamete containing one of the chromosomes produces a zygote with three such chromosomes; the normal pair plus an extra one. This condition is called **trisomy**. Fusion of a gamete with none of the homologous chromosomes with a normal gamete gives rise to an individual with only one of this particular type of chromosome in each cell. This condition is called **monosomy**.

Non-disjunction can cause various chromosome abnormalities in humans. For example, Turner syndrome is an example of monosomy in the sex chromosomes. Foetuses with 22 normal pairs of

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autosomes and a single Y chromosome never survive to birth. However, children may be born with 22 normal pairs of autosomes and a single X chromosome. Such individuals have the genetic constitution X0. They are females and occur with an incidence of approximately 4 in 10 000 live-born girls. Most of the phenotypic effects of Turner syndrome are minor, but the person is infertile. Individuals are usually shorter than normal and have a characteristic webbed neck. Oestrogen replacement therapy can allow normal pubertal development, and growth can be stimulated with growth hormone.

Approximately two in every thousand men have a trisomy in sex chromsomes (XXY), which is known as Klinefelter syndrome. This may result either from the fusion of a Y sperm with an XX egg or from the fusion of an XY sperm with an X egg. Although XXY individuals are phenotypically men, they have very small genitals and are infertile. In addition, they may develop breasts, although testosterone therapy at puberty can reduce this effect.

Down syndrome is a trisomy caused by the presence of an extra copy (i.e. a total of three copies) of chromosome 21 (one of the smallest chromosomes) in every cell. Children with Down syndrome vary in their symptoms, but most show moderately to severely delayed development, characteristic almond-shaped eyes, a round face, shortened body parts, loose joints, and weak muscles and muscle reflexes. About 40% of children with Down syndrome develop heart defects, and they are more susceptible to infections, both of which can cause their lives to be shorter. They are usually affectionate, cheerful people, often deriving great pleasure from music and dancing. Down syndrome is an example of autosomal trisomy, because a *non-sex* chromosome is added (Figure 4.21). It is the most common autosomal trisomy in humans.

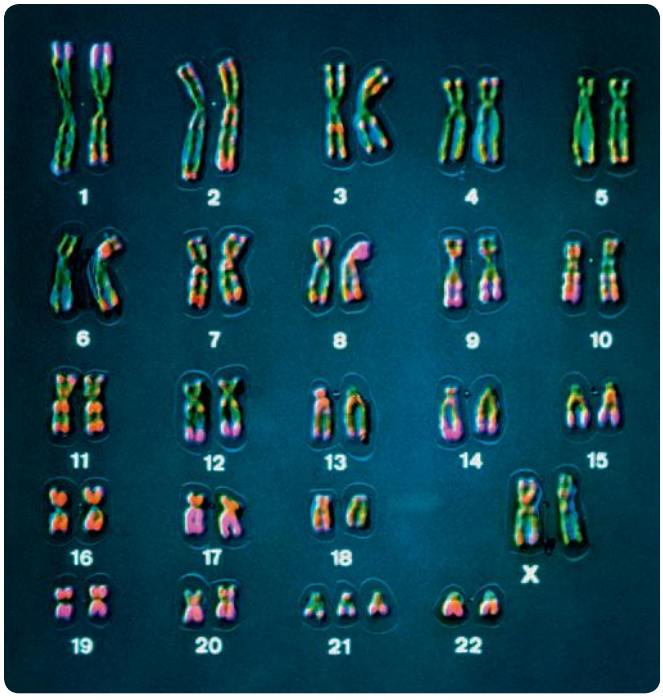

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FIGURE 4.21 False-colour karyotype from a female with Down syndrome. The syndrome is the result of there being three copies of chromosome number 21. This condition is also known as trisomy 21.

Key concept

Chromosome mutations relate to entire chromosomes. Mutations that change the number of chromosomes lead to polyploidy and aneuploidy.

Variations in chromosome structure

Changes in chromosome structure largely come about due to the occurrence of two or more double-strand breaks in chromosomes and the rearrangement of the broken segments of the chromosomes. Some of these breaks occur naturally during meiosis as the chromosomes entangle around one another, cross over and move apart. Others occur because of exposure to mutagens that accelerate the rate of double-strand breaks. The breaks are normally repaired, but occasionally mistakes are made in the way the segments are relocated in the repaired chromosomes. There are several classes of these chromosomal rearrangements: deletions, inversions, translocations and duplications (Figure 4.22).

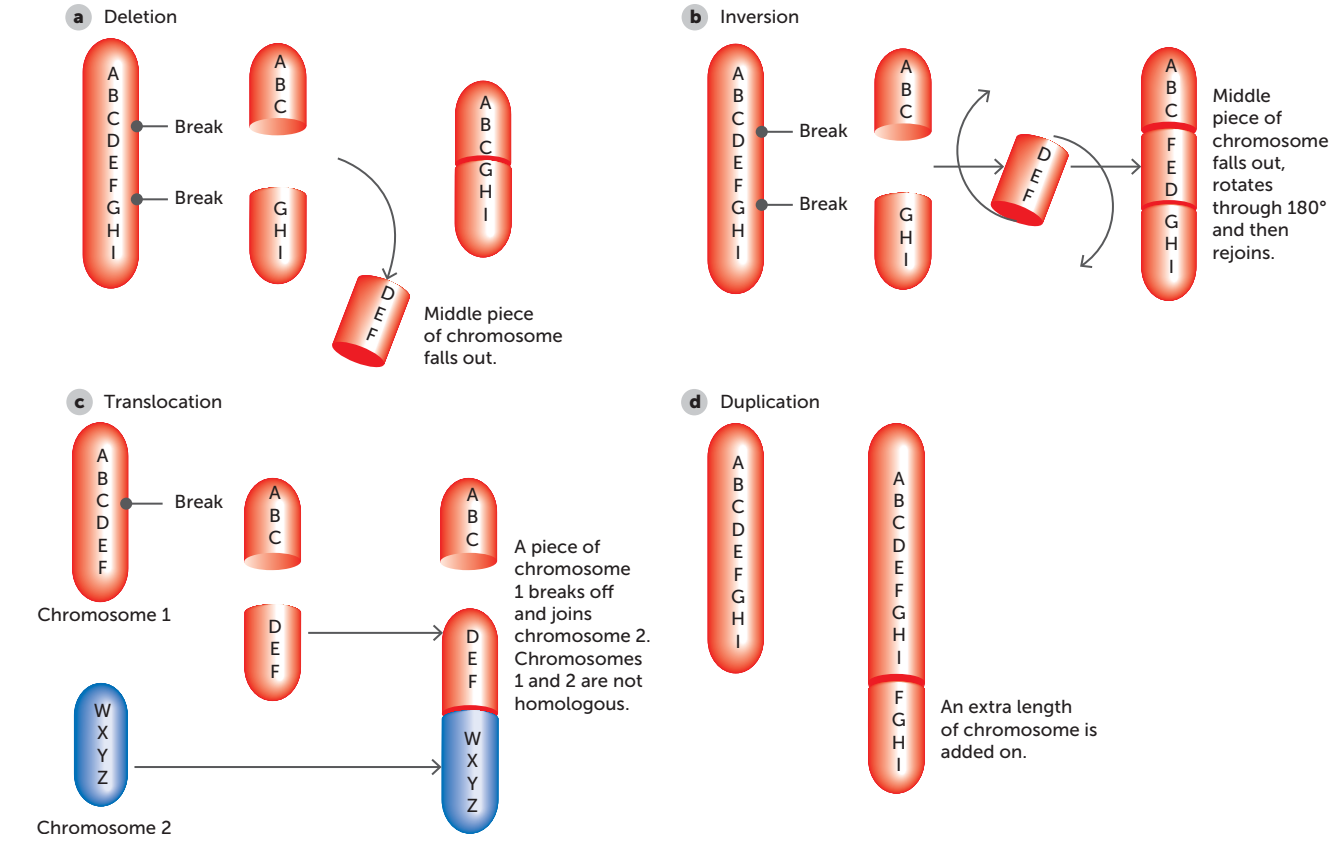

FIGURE 4.22 Abnormalities caused by chromosomal mutations may arise by **a** deletion, **b** inversion, **c** translocation or **d** duplication.

Deletions

A chromosome may undergo double-strand breaks at two positions, and the section in between may drop out, removing all its genes with it. If the two ends then re-join, a shorter chromosome results with a segment missing. This is called a chromosome deletion (Figure 4.22a). As it leads to an absence of certain genes, it can have a profound effect on the development of an organism. All but the shortest deletions are usually fatal, and the few organisms that survive usually suffer from adverse effects.

Williams syndrome is an example of a condition that arises because of a deletion event that affects about 1 in 10 000 people. Patients are characterised by certain physical (Figure 4.23) and temperamental features, including an unusually cheerful and affectionate disposition, and an exaggerated predilection for music and dance. The syndrome is associated with an unusual

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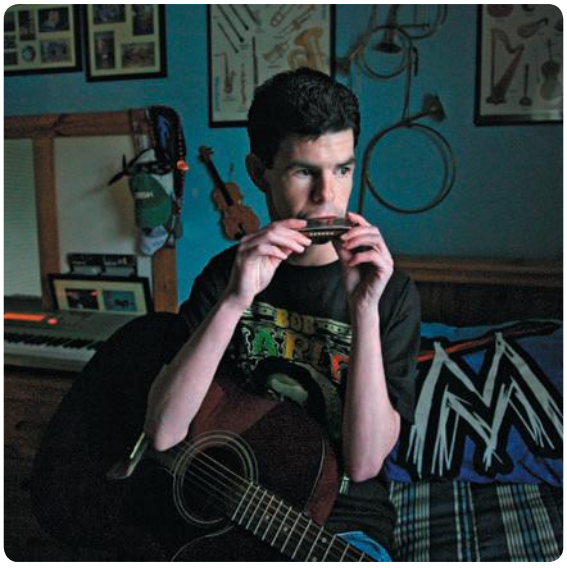
development of the nervous system, developmental delay and life-threatening cardiovascular problems. Williams syndrome is caused by the deletion of around 1.5 million nucleotide base pairs from one copy of chromosome 7. The DNA segment carries between 15 and 25 genes of known and unknown function. The syndrome demonstrates that both copies of one or more of these genes are required for normal development.



ALAMY STOCK PHOTO/ZUMA PRESS INC

FIGURE 4.23 A person with Williams syndrome is characterised by a facial appearance with a low nasal ridge.

Inversions

Another kind of chromosomal rearrangement occurs if a chromosome breaks in two places and the segment in the middle rotates through 180° before being re-joined within the chromosome, reversing the normal sequence of genes (Figure 4.22b). This is called an inversion. The effects of inversions are usually less dramatic than other types of chromosomal changes, because genes have been neither gained nor lost, and the genes within the inverted segment can still function normally. The inversion may, however, disrupt a gene in which it occurs or cause two different genes to become fused together. Also, if the chromosomes do not align properly for meiosis, the affected individual may have reduced fertility.

Translocations

The devil is in the details
Read about Tasmanian devil facial tumour and translocation

Sometimes a section of one chromosome breaks off and reattaches to another chromosome. This is known as a translocation (Figure 4.22c). An example of a translocation in humans is when a segment of chromosome 8 ends up within chromosome 14, or vice versa. Normal control over the genes in that segment is lost, often resulting in a form of cancer. In addition to aneuploidy, a Tasmanian devil facial tumour cell karyotype shows translocation of chromosome 5.

Duplications

A duplication occurs when an extra copy is made of a section of a chromosome and inserted either into the same chromosome or into another chromosome (Figure 4.22d). Gene sequences can be replicated several times, sometimes thousands of times. Like other chromosomal abnormalities that change the number of copies of particular genes, duplications of chromosomes are frequently harmful. However, on occasions they may be advantageous. The various genes that control the different haemoglobins produced in human red blood cells are thought to have arisen by duplications. Changes to chromosome structure such as inversion, deletion, translocation and duplication of chromosome segments are another cause of genetic variation.

Key concept

Structural mutations to chromosomes include deletions, inversions, translocations, duplications and frameshift mutations.

TABLE 4.3 Major classes of mutations, their types and their effects on DNA

CLASS OF MUTATION	TYPE OF MUTATION	DESCRIPTION
Point mutation	Substitution	One base is replaced by another during replication, as is its base pair in the corresponding position on the complementary strand.
	Insertion	One or more extra nucleotides are inserted into replicating DNA, often resulting in a frameshift.
	Deletion	One or more nucleotides is 'skipped' during replication or otherwise cut out, often resulting in a frameshift.
Chromosomal mutation	Inversion	One region of a chromosome is flipped and reinserted.
	Deletion	A region of a chromosome is lost, resulting in the absence of all the genes in that area.
	Translocation	A region from one chromosome is aberrantly attached to another chromosome.
	Duplication	A region of a chromosome is repeated, resulting in an increase in copies of the genes in that region.

Question set 4.6b

REMEMBERING

1. Define karyotype.
2. Describe the differences between:
   - **a** haploid and diploid
   - **b** monoploid and haploid
   - **c** monoploid, diploid and polyploid
   - **d** diploid and aneuploid.
3. Describe four types of DNA structural rearrangements that result in chromosomal abnormalities.

UNDERSTANDING

4. Draw an annotated diagram of a diploid cell with four chromosomes undergoing meiosis. Show two ways that non-disjunction can occur. Indicate the kind of chromosome anomalies that can arise in a zygote formed by fertilisation between each of the resulting gametes and one normal gamete.
5. Draw an annotated diagram of two chromosomes, showing that one of them has had two double-strand breaks. Draw the possible chromosomal rearrangements that might occur when the fragments of the broken chromosome are re-joined.
6. Create an electronic or hard copy mind map to summarise the gene and chromosome mutation types you have learned about. You could use Prezi or MindMeister.
7. Defend or refute the statement, 'Aneuploidy is always deleterious', and explain your reasoning.

APPLYING

8. How can karyotypes be used to determine variation in a population?

4.7 SEXUAL REPRODUCTION INCREASES VARIATION

Variations in the genotype of offspring arise as a result of the processes of meiosis, including crossing over, random assortment of chromosomes, and fertilisation.

The genetic component of variation is predominantly determined by alleles. Alleles are transmitted from generation to generation through the production of gametes by meiosis, and the union of those gametes through fertilisation. During meiosis, homologous chromosomes pair and then randomly move to different daughter cells (**independent assortment**). This results in gametes

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with different combinations of parental chromosomes and therefore different combinations of parental alleles. When homologous chromosomes pair up in the first division of meiosis, they sometimes exchange segments with one another. This crossing over further rearranges the combinations of inherited alleles available on each homologous chromosome. Fertilisation comes about by the union of two random gametes, one from each parent, providing even further variation in the possible combinations of inherited alleles.

Crossing over

Crossing over is the swapping of alleles that occurs in meiosis during prophase I only. During the formation of egg and sperm cells in meiosis, paired maternal and paternal homologous chromosomes align so that corresponding DNA sequences from the paired chromosomes are able to cross over one another.

Crossing over is important for genetic variation, because it allows the exchange of alleles between the maternal and paternal homologous chromosomes (non-sister chromatids). This forms chromatids with new combinations of alleles (this can be referred to as recombination of linked genes). Chromatids that have a combination of alleles different from that of either parent are called recombinants (Figure 4.24). It is also important to note that crossing over occurs at a random point, and more than one **chiasma** can form per homologous pair. The chiasmata are the points of contact between two (non-sister) chromatids belonging to a set of maternal and paternal homologous chromosomes.

At the end of meiosis, there are four possible gametes. The four haploid cells contain chromosomes that are genetically different from the parent cells and from one another.

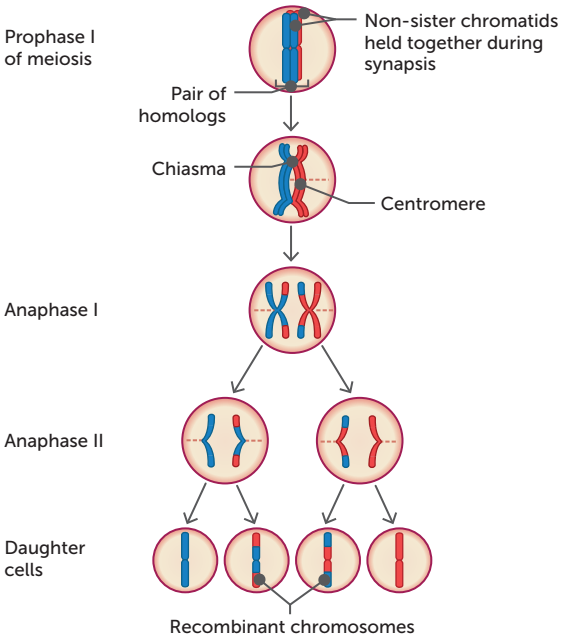

FIGURE 4.24 Recombinant chromosomes

Independent assortment and random segregation

The Law of Independent Assortment refers to the random orientation of maternal and paternal homologous chromosomes at the equator during metaphase I. Each member of the homologous pair is randomly orientated towards one pole or the other, and each pair is unaffected by the orientation of any other homologous pair. In Figure 4.25 (page 109), the left-hand possibility in gamete production shows the paternal chromosome (shown in blue) orientated to the left in two homologous pairs of chromosomes. In the right-hand possibility, one paternal chromosome is orientated to the left and the other to the right.

An allele on one chromosome has an equal chance of being paired with, or separated from, any allele on another chromosome (their inheritance is independent). Since the homologous pairs of chromosomes are orientated randomly at the equator, maternal and paternal homologues can orientate towards either pole. The number of possible orientations is equal to 2 raised to the power of the number of chromosome pairs. For example, for a haploid number of n, 2^n is the number of possible outcomes. For humans, the possible number of combinations is 2^{23}. This means that there are over 8 million possible combinations of alleles just through the random orientation of the homologous chromosomes. If we add the effects of crossing over, the number of combinations increases even more.

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During anaphase I, the randomly lined up maternal and paternal homologous chromosomes move to opposite poles of the cell. This random separation of a pair of homologous chromosomes is described as the Law of **Random Segregation**. Each gamete ends up with a random selection of maternal and paternal chromosomes. The chromosomes have segregated (separated) randomly into the four gametes (Figure 4.25).

Independent assortment
Study independent assortment by watching the animation.

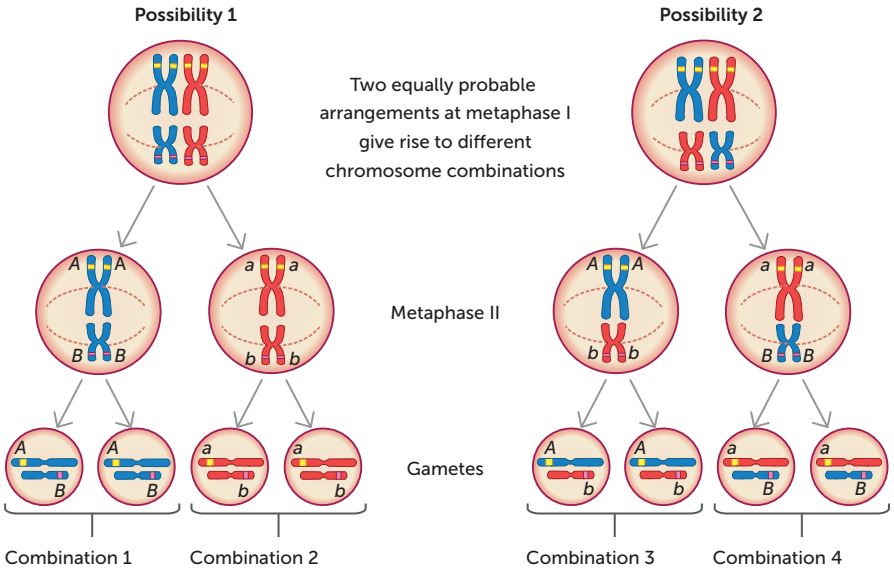

FIGURE 4.25 Independent assortment and random assortment lead to different combinations of chromosomes in gametes.

Random fertilisation

Fertilisation is the union of haploid male and female gametes during sexual reproduction to produce a diploid zygote. The random union of gametes is known as **random fertilisation**. Random fertilisation can lead to variation. Fertilisation brings together chromosomes from two different parents, creating new combinations of alleles in the offspring. When a female gamete is made during meiosis, it receives 50% of the mother's genetic information. The same is true for a male gamete. Due to independent assortment and random distribution of the chromosomes when the cells split during meiosis, each gamete has a different combination of chromosomes from that of other gametes. Therefore, each gamete has a unique set of alleles. Fertilisation promotes variation because a male gamete can fertilise any of the female gametes, resulting in a unique combination of the maternal and paternal genes. An additional source of variation in the possible gamete combinations is the random selection of a mate. The offspring produced in sexual reproduction are genetically different to one another and to their parents. Sexual reproduction results in variation within a population because it involves the mixing of genetic information.

Fertilisation can occur internally (as in humans) or externally (as in the majority of corals). Gametes contain recombinations of genetic material, and the different gamete combinations possible during fertilisation increases variation.

Key concept

Sexual reproduction contributes to variation. Crossing over and independent assortment occur during meiosis to produce variety in gametes, and a random selection of these gametes will be randomly fertilised. The result of fertilisation is an individual with a different genotype to that of its parents.

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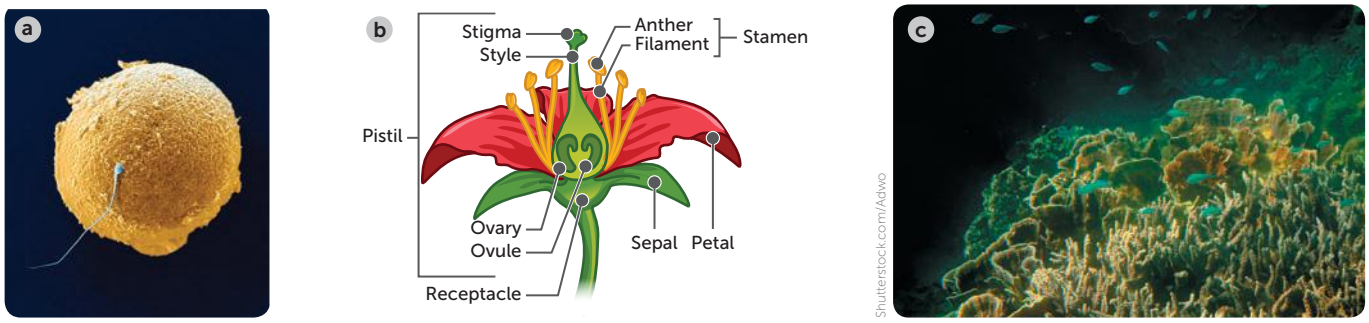

FIGURE 4.26 Fusion of male and female gametes occurs in sexually reproducing species. **a** A coloured scanning electron micrograph of a human sperm and egg. **b** Pollen contains the male gametes of a flowering plant and is found on the stamen, or male structure, of a flowering plant. Ovules contain the female gametes of a plant and are found in the pistil, or female structure, of the plant. The pollen travels to the stigma (i.e. 'pollination' occurs), then fertilisation takes place in the ovule. Variation is enhanced in plants if the flower does not self-pollinate. **c** Coral at Ningaloo Reef, WA, sexually reproduce 7–10 days after the full moon in March. The coral polyps spawn – that is, they release eggs and sperm into the water – at the same time. The eggs and sperm randomly fertilise to form larvae known as planulae.

Question set 4.7

REMEMBERING

1. Recall the function, process and products of meiosis.
2. Define:
   - **a** crossing over
   - **b** chiasmata
   - **c** independent assortment
   - **d** random segregation
   - **e** fertilisation.

UNDERSTANDING

3. Draw an annotated diagram of a diploid cell with two homologous chromosomes undergoing crossing over.
4. Draw an annotated diagram showing how independent assortment contributes to variation in a population.

APPLYING

5. Explain why sexual reproduction leads to more variation in a population than asexual reproduction.

4.1 APPLICATION

Editing the epigenome

Researchers at the Harry Perkins Institute of Medical Research are using advanced genomic, molecular, genetic and computational techniques to study the epigenome, including next-generation sequencing technologies to generate whole-genome high-resolution maps of the epigenome and associated molecular processes. Professor Ryan Lister hopes to develop molecular tools for editing the epigenome.

Questions

1. What is an epigenome?
2. What is DNA methylation and why is it important?
3. 'Research aims to elucidate the mechanistic underpinnings of how the epigenome is established and dynamically modified, and how it affects the cellular readout of the underlying genetic information, and to develop molecular tools for editing the epigenome.' What does this mean in terms of treatment of genetic diseases?

CHAPTER 4 INVESTIGATION

Developed by Southern Biological

The effect of UV light on *Saccharomyces cerevisiae*

4.1 INVESTIGATION

Background

We classify the broad spectrum of electromagnetic radiation from the sun into segments according to the effects we experience. For example, the warm sensation of sunshine on our skin is caused by invisible infrared radiation with wavelengths ranging from 700 nm to 1 000 000 nm (1 mm). Visible light is comprised of wavelengths of between 400 nm (violet) and 700 nm (red). Radiation with a wavelength shorter than 400 nm but longer than 10 nm is classified as ultraviolet (UV) radiation. Radiation with a wavelength shorter than 10 nm is classified as X-rays.

Some exposure to UV radiation is necessary for humans to produce vitamin D, but a careful balance is required, because X-rays and UV radiation are destructive of many biological molecules, including DNA. Fortunately, Earth's atmosphere acts as a protective screen and filters out almost all the sun's radiation that has wavelengths shorter than 290 nm. Nevertheless, the narrow UV band from 290 nm to 400 nm that can penetrate the atmosphere and reach Earth's surface is capable of causing photochemical damage to DNA that can lead to skin cancer, so it is important to avoid over-exposure. As a defence against UV exposure, most organisms that are subject to the sun's rays have evolved to incorporate some level of DNA repair in their cellular mechanisms. This confers a limited amount of inherent UV resistance.

Aim

To determine how UV radiation can be destructive of many biological molecules.

Time requirement

55 minutes

Materials

- UV-sensitive yeast starter plate
- Wild-type yeast starter plate
- 8 sterile swabs
- 8 YED agar plates
- 4 plastic pipettes
- Sterile water
- 2 sterlie inoculation loops
- 2 sterile culture tubes
- Ethanol or bleach
- Bunsen burner
- Adhesive tape
- Permanent marker
- PPE: lab coats, safety glasses, disposable gloves

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
While lab strains are usually harmless, fungi may cause disease, so assume them to be pathogenic.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end of activity. Decontaminate benches before and after activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Disposable gloves may pose an allergy risk.	Use a type of glove that has no allergy risk and is suitable to the chemicals being used.

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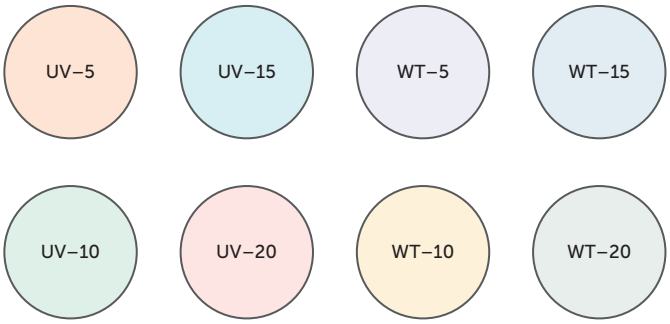
Procedure – inoculation of exposure plates

To use aseptic technique, wipe your bench down with ethanol (or bleach) and keep your work near the Bunsen burner to waft potential contaminants away from your materials.

1 Collect 8 YED agar plates and label them as follows using a permanent marker.



Key
UV = UV-sensitive yeast (mutated strain)
WT = Wild-type yeast
Number = Time plate will be exposed to sunlight

FIGURE 4.27 Labelled petri dishes

2 Use a plastic pipette to place 1 mL of sterile water into a sterile culture tube.
3 Use a sterile inoculation loop to carefully scrape a single colony of the UV-sensitive yeast from the starter plate.
4 Select a large colony (>4 mm in diameter), or if the colonies are small, scrape up two (or even three) onto the loop.
5 Place the loop in to the sterile tube and spin/swirl it to transfer the yeast into the sterile water.
6 Visually check that the cell mass has transferred from the loop into the water.
7 Immediately, use a 1 mL plastic pipette to pump the liquid to distribute and suspend the yeast cells in the water. Avoid introducing air bubbles or splashing the liquid up the sides of the tube. When you have finished, hold the tube up to the light to check that there are no visible lumps or particles in the water.
8 Dip a sterile swab into the yeast suspension and, as you withdraw it, press it against the sides of the tube to squeeze out excess water. It should come out moist but not dripping.
9 Using an aseptic technique, swab the surface of a YED agar plate in three directions to inoculate for a lawn culture.
10 Immediately cover the plate to shield it from light, and allow it to rest (right-way up) for a period of at least 15 minutes and up to 1 hour. This allows the moisture from the swab to be absorbed by the agar.
11 Repeat steps 8–10 for the remaining three UV-sensitive yeast plates. Then repeat this procedure for the four plates using the wild-type yeast.

Procedure – exposure to sunlight

1 State your hypothesis for this experiment.
2 After the post-inoculation resting period, expose one inoculated plate from each strain to direct sunlight for 5, 10, 15 and 20 minutes, respectively.
3 Immediately after exposure, incubate the plates in darkness for 48 hours at 30°C or 4 days at room temperature.
4 For best results, follow these guidelines:
- Keep the plate shielded from light until the last moment.

- Use adhesive tape to attach the lid of the petri dish to the base, but do not allow the tape to extend onto the surface of the lid where it would absorb UV light and shield the yeast from exposure.
- Orientate the plate so the lid is pointing directly at the sun. Aim to minimise the size of the shadow. If the angle between the sun's rays and the lid is small, most of the UV light will be reflected and the effectiveness of the exposure will be reduced.
- Schedule the investigation at a time of year when you can be sure of bright, sunny conditions.
- After the incubation period, observe and compare the level of coverage between the plates.

Results

Copy and complete the table below with the results of your experiment. Use the key below to indicate the level of coverage of the yeast on each agar plate.

Key

+++	High coverage
++	Medium coverage
+	Low coverage
–	No coverage

TABLE 4.4 UV exposure results

EXPOSURE TIME (minutes)	UV-SENSITIVE YEAST COVERAGE	WILD-TYPE YEAST COVERAGE
0		
5		
10		
15		
20		

1. Compare the results of your UV-sensitive yeast sample with those of the wild-type yeast sample. What differences do you observe?
2. What conclusions can you draw from this data?
3. Draw a graph of your results.

Discussion

1. What is your independent variable?
2. What is the range of your independent variable?
3. What is your dependent variable?
4. What are your control variables and how did you control them?
5. What type of mutation does the UV-sensitive yeast display?
6. Compare your results with those of others in your class. Were the results consistent?
7. Did your experiment support or refute your hypothesis, or were your results inconclusive?
8. Based on your findings, how does UV light impact the two different yeast strains? Do they differ? Explain why, if they do.

Taking it further

To protect our skin from harmful UV rays, we apply different sunscreens with different sun protection factor (SPF) values. Do these values have any merit, and are commercially produced screens any better

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WS
Chapter 4 Activity sheet

CHAPTER 4 SUMMARY

- Phenotype is affected by the genotype of the individual and the environment in which the individual lives. Variation in DNA, genes and chromosomes causes variation between individuals and species.
- Environmental factors include internal (e.g. hormones), external (e.g. temperature) and epigenetic factors that affect gene expression without changing the DNA sequence.
- Mutations are permanent changes to a DNA sequence. Mutations can occur in somatic cells where they are not passed on to the next generation) and in germline cells (through which they can be passed on to the next generation).
- Mutations can be caused by errors during DNA replication (spontaneous mutations); errors during cell division (crossing over); or the action of physical, chemical or biological mutagens.
- Mutations can be classified as point (including single nucleotide polymorphism, SNP) or chromosome (large-scale change) mutations.
- Point mutations include substitutions (synonymous, missense, nonsense) and additions or deletions (frameshift mutations) and can be neutral, harmful or beneficial to the survival of the organism.
- Chromosome mutations include variations in the number of chromosomes (polyploidy and aneuploidy) and variations in the structure of chromosomes (deletions, inversions, translocations, duplications and frameshift mutations).
- Sexual reproduction produces variation in offspring through crossing over of homologous chromosomes, independent assortment and random segregation, and the fertilisation of random gametes.

CHAPTER 4 GLOSSARY

Allele One of various versions of the same gene (at the same locus) distinguished by small differences in the DNA sequence

Aneuploidy Describes a genome that varies from the conventional genome through the loss or addition of one or a few chromosomes

Apoptosis A programmed series of events that leads to cell death as a result of the dismantling of the internal contents of the cell by various enzymes, including caspases

Behaviour Responses and reactions of an organism in particular situations

Beneficial mutation A mutation that increases an organism's chances of survival and reproduction

Chiasma The point of contact between two (non-sister) chromatids belonging to a set of maternal and paternal homologous chromosomes where crossing over may occur

Chromatin The complex of proteins and DNA found in eukaryotic non-dividing cells

Cloning vector In cloning, the vector is the DNA molecule that is used to carry the cloned piece of DNA

Codon A set of three consecutive nucleotides found in a DNA or an mRNA molecule; it carries a code for a specific amino acid

Crossing over An event during meiosis, in which homologous chromosomes (non-sister chromatids) exchange alleles (genetic segments) with one another

Deleterious mutation A mutation that decreases an organism's chances of survival and reproduction

Deletion mutation A mutation in which one or more nucleotide pairs have been lost from a segment of DNA

Diploid (2*n*) Describes a cell or organism that has a genome that contains two copies of each chromosome, represented by 2*n*

Double-strand break A mutation involving breaks in the sugar–phosphate backbones of both DNA strands at the same nucleotide pair, resulting in the complete breakage of a chromosome

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Epigenetics The study of inheritable, but reversible, changes caused by chemicals that control the activity of DNA; it involves activation and deactivation of genes, without any change in the DNA sequence or code

Expressed Describes a gene that has been read, transcribed and translated into a protein

Fertilisation The union of haploid male and female gametes during sexual reproduction to produce a diploid zygote; the random union of gametes is known as random fertilisation

Frameshift mutation A mutation that changes the reading frame used in translation, during polypeptide synthesis

Gamete A sex cell; it can be a male or female sex cell and has a haploid number of chromosomes

Gene A unit of heredity that transmits information from one generation to the next; a segment of DNA that codes for a polypeptide

Genetic code The term used for the way that the four nitrogenous bases of DNA (adenine, thymine, guanine and cytosine) are ordered and contain information to direct the creation of specific proteins

Genome All of the genetic material contained in an organism or a cell; it includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

Genotype The specific combination of alleles for each gene locus that belongs to an individual or cell

Germline cell The cell line in eukaryotic organisms from which the gametes are derived

Haploid (*n*) Describes a cell or organism that has a genome that contains one copy of each chromosome; represented by *n*

Heredity The study of inheritance; the genetic transmission of characteristics from one generation to another

Homologous chromosome A pair of chromosomes that have the same size and shape; they have genes at the same locations; one is maternal and one is paternal

Horizontal gene transfer The process by which genetic material from one organism becomes incorporated into the genome of another organism

Independent assortment The random orientation of maternal and paternal homologous chromosomes at the equator during metaphase I; the orientation of each homologous pair is randomly to one side or the other, and each pair is unaffected by the orientation of any other homologous pair

Insertion mutation A mutation in which one or more nucleotide pairs have been added to a segment of DNA

Intraspecific variation Differences between individuals of the same species

Karyotype A display that presents the number and appearance of the chromosomes of an organism or cell as observed at metaphase

Meiosis A type of cellular division in sexually reproducing organisms that involves two rounds of cell division, but only one round of DNA replication; during meiosis, the chromosome number of a cell is halved

Missense mutation A gene mutation that results in one amino acid being replaced by another amino acid in the encoded protein

Monoploid (1*n*) Describes a cell or organism that has a functional genome consisting of one copy of each chromosome, represented by 1*n*

Monosomy The condition in which somatic cells of an organism are missing one copy of a particular chromosome

Mutagen An agent capable of inducing mutations

Mutant A cell or organism that bears a mutation

Mutation A permanent change in the DNA sequence of a gene; a source of new alleles in a population's gene pool; the process of generating a mutation

Mutation rate The number of changes per gene copy in a population over a period of time

Neutral mutation A mutation that has no effect on an organism's chances of survival and reproduction

Non-disjunction The failure of sister chromatids in mitosis or homologous chromosomes in meiosis to separate and go to opposite poles

Nonsense mutation A mutation in which a codon for an amino acid is changed to one

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that codes for a stop codon, terminating translation

Parthenogenesis The production of offspring, usually from a female gamete without the requirement for fertilisation

Phenotype The actual observable form taken by a specific feature in a particular individual, based on their genotype and influenced by the environment; it can be used in reference to particular traits or characteristics or to the overall form of an individual

Plasmid A small circular piece of DNA (found in bacteria) that is able to replicate independently of the cell's chromosomes; engineered plasmids can carry antibiotic-resistance markers

Point mutation A mutation that affects a single base-pair within a gene

Polyploidy A cell or organism with a genome comprising three or more copies of each chromosome, represented by $3n$, $4n$, $5n$, $6n$ etc.

Random fertilisation The union of a male gamete and a female gamete, both haploid, which results in a diploid cell called a zygote; it is random because there is no way of knowing which two gametes, each genetically unique, will form the zygote

Random segregation The phenomenon that starts during anaphase I, when the randomly lined-up maternal and paternal homologous chromosomes move to opposite poles of the cell, illustrating the Law of Random Segregation; each gamete ends up with a random selection of maternal and paternal chromosomes

Silent mutation See **synonymous mutation**

Single nucleotide polymorphism (SNP) A single nucleotide difference that occurs at a given position in the genomes of two or more individuals

Somatic cell A body cell that is not a germ cell

Species A group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same

Spontaneous mutation A mutation occurring in the absence of exposure to mutagens

Substitution mutation A mutation in which a single nucleotide is swapped for another in the original gene sequence

Synonymous mutation A mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a 'silent' mutation

Trisomy A condition in which somatic cells contain three copies of a particular chromosome

Variation The diversity of genetic and phenotypic traits within and between populations

CHAPTER 4 REVIEW QUESTIONS

Remembering

1 Identify which kind of phenotypic variation is represented by each of the following.
   - **a** You enjoy solving mathematical puzzles, whereas your friend is frustrated by them.
   - **b** The daily amount of milk produced varies between different individuals of a particular variety of cow (*Bos taurus*).
   - **c** The girth of mature mountain ash (*Eucalyptus regnans*) trees varies.
   - **d** Some cowpeas (*Vigna unguiculata*) grow better in acidic soils than others.
   - **e** The presence or absence of a hydroxyl (OH) group on the anthocyanin pigment differs between individual corn plants (*Zea mays*).

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Understanding

2 Figure 4.28 is a representation of segments of chromosomes with genes numbered along their lengths. Identify the mutation that has occurred in each of these structural rearrangements from the original for each of **a** to **d**.

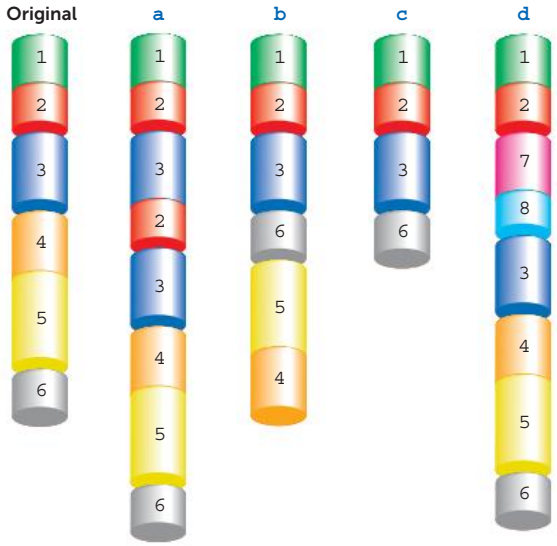


FIGURE 4.28 Chromosomal mutations

3 Discuss the relationship between SNPs (substitutions) and synonymous, missense and nonsense mutations.

Applying

4 Identical twins, like the French bulldog puppies in Figure 4.29, usually have the same genotype.

Alamy Stock Photo/Nature Picture Library

FIGURE 4.29 French bulldog puppies

Explain why one may grow to be a larger size than the other.

5 What might account for the fact that most forms of aneuploidy are rarely, if ever, observed in humans?

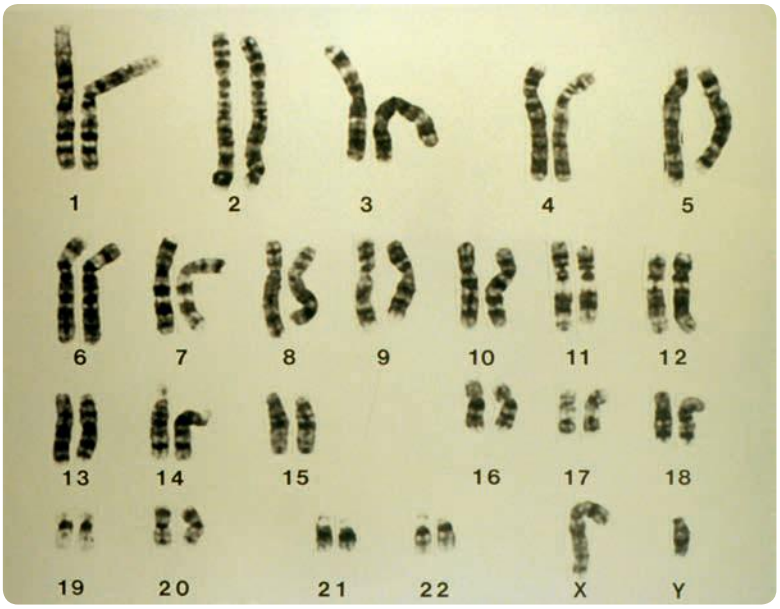
6 A transposable element consisting of precisely 270 nucleotide pairs lands in the intron of a functional gene without affecting the splicing sites for the transcribed RNA.

- **a** What kind of genetic mutation does this represent?
- **b** Describe the effect of the extra nucleotides on the protein sequence.
- **c** Would this be likely to be a neutral, beneficial or deleterious mutation?

7 Compare the four human karyotypes **i** to **iv** in Figure 4.30.

- **a** Determine which of the four is a normal karyotype and identify the sex of the individual.
- **b** Determine the aberration in each of the other three karyotypes.
- **c** Explain how the three aberrations could have come about.

i



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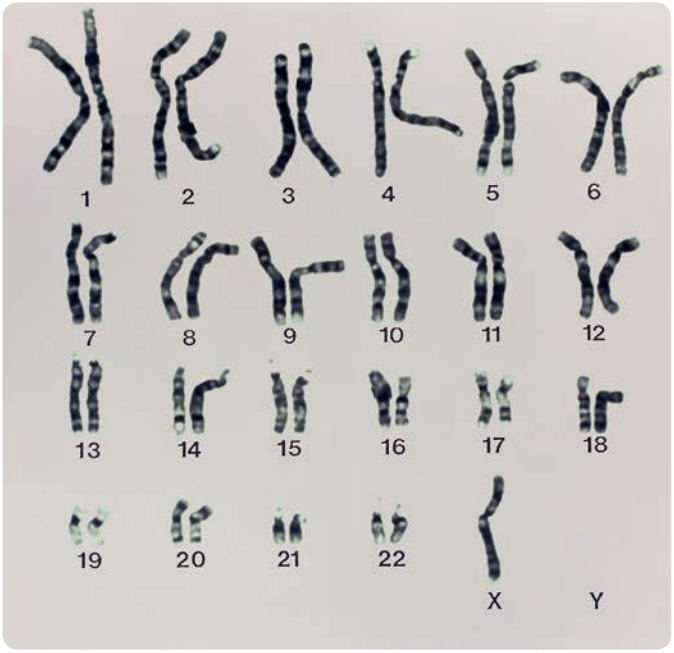

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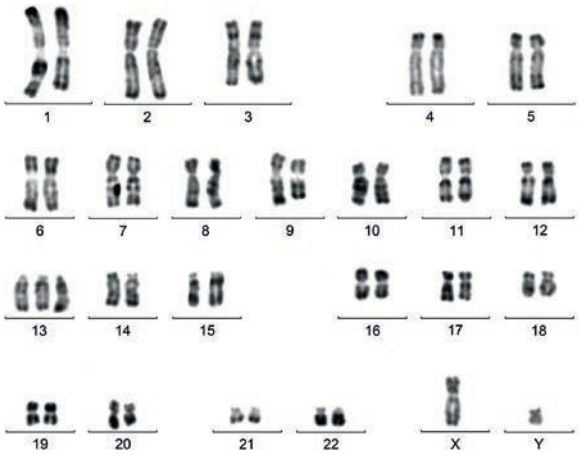

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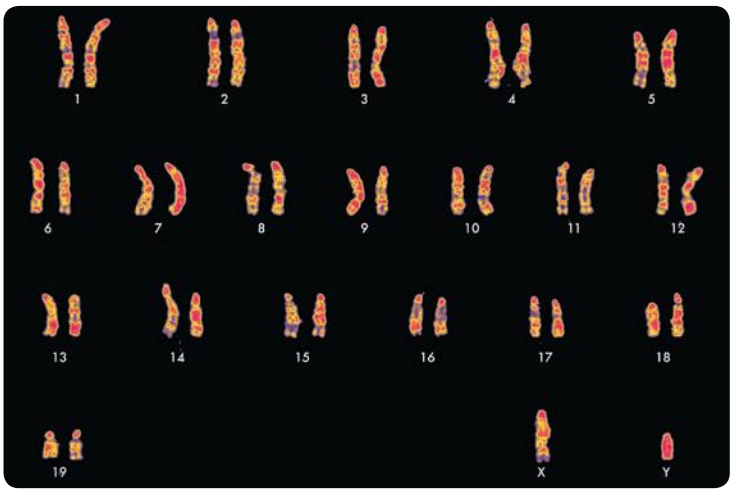

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FIGURE 4.30 Four human karyotypes

8 Copy and complete Table 4.5 using the information provided in Table 4.6. Note that more than one type of mutation may have the same effect on the protein.

TABLE 4.5 Four human karyotypes

GENETIC MUTATION	AMINO ACID	TYPE OF GENETIC MUTATION	EFFECT ON PROTEIN
GTCCCA ↓ GTCCCT	Valine–Proline ↓ Valine–Proline	Substitution	Synonymous
TCAATA ↓ TAATA	Serine–Lysine ↓		
AGAGGT ↓ AGATGT	Arginine–Glycine ↓		
GCAAGA ↓ GAAAGA	Alanine–Arginine ↓		
CAGTAC ↓ CACGTAC	Glutamine–Tyrosine ↓		

TABLE 4.6 Properties, names and DNA codons for each of the 20 amino acids

CHARACTERISTICS	NAME	DNA CODONS
Small, hydrophobic	Glycine	GGT, GGC, GGA, GGG
	Alanine	GCT, GCC, GCA, GCG
	Valine	GTT, GTC, GTA, GTG
	Leucine	TTA, TTG, CTT, CTC, CTA, CTG
	Isoleucine	ATT, ATC, ATA
Cyclic	Proline	CCT, CCC, CCA, CCG
Bulky, hydrophobic	Phenylalanine	TTT, TTC
	Tyrosine	TAT, TAC
	Tryptophan	TGG
Sulfur-containing, hydrophobic	Methionine (START)	ATG
	Cysteine	TGT, TGC
Hydrophilic	Serine	TCT, TCC, TCA, TCG, AGT, AGC
	Threonine	ACT, ACC, ACA, ACG
	Asparagine	AAT, AAC
	Glutamine	CAA, CAG
Positively charged, hydrophilic	Aspartic acid	GAT, GAC,
	Glutamic acid	GAA, GAG
Negatively charged, hydrophilic	Histidine	CAT, CAC
	Lysine	AAA, AAG
	Arginine	CGT, CGC, CGA, CGG, AGA, AGG
	STOP	TAA, TAG, TGA

Analysing

9 List all the codons that could result from a synonymous mutation of GGG. What observation can you make about which of the three nucleotides in the codon is most prone to being mutated?

10 An exceptionally large plant with enlarged fruit grows among a natural population. Discuss what genetic change might have occurred in this individual and describe how you could test it to find out.

11 'X-ray imaging of a newborn child and a fully grown adult are equally risky.' Consider whether you agree or disagree with the statement and explain your reasoning.

Evaluating

12 Polycyclic aromatic hydrocarbons are a diverse collection of compounds produced by combustion, occurring, for example, in cigarette smoke. With reference to effects, mutations, mutation rates and level of exposure, provide an explanation for the statistical link between cigarette smoking and the increased incidence of lung cancer.

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13 Imagine a situation in which a child of dark-skinned parents has inherited a mutated form of a gene that confers light skin pigmentation. Predict whether this mutation would be neutral, beneficial or deleterious if the individual is located in the Arctic Circle, rather than in equatorial Africa, and explain your reasoning. Discuss how, if at all, your interpretation of 'neutral', 'beneficial' and 'deleterious' is influenced by the individual's environment.

Creating

14 Design a poster to show how an addition mutation can lead to a frameshift.

15 Construct a concept map to summarise the factors and processes involved in each of the three main mechanisms of variation.

Reflecting

16 What are some of the potential mutagens you encounter in your daily life, and how might you reduce your exposure to some of them?

PRACTICE EXAM QUESTIONS

1 The diploid number of chromosomes in the chimpanzee is 48. A chimpanzee with stunted growth and other abnormalities was found to have 49 chromosomes. The most likely source of the extra chromosome in this chimpanzee is:

- **A** a viral infection in the chimpanzee
- **B** a viral infection in one of the parents of the chimpanzee
- **C** an error in meiosis in the chimpanzee
- **D** an error in meiosis in one of the parents of the chimpanzee.

[Q18 2017 SCSA]

2 Crossing over is the:

- **A** exchange of alleles between homologous chromosomes
- **B** exchange of alleles between non-homologous chromosomes
- **C** segregation of homologous chromosomes to different poles
- **D** segregation of non-homologous chromosomes to different poles.

[Q27 2017 SCSA]

3 X-radiation (X-rays) is an agent that:

- **A** repairs DNA and decreases the mutation rate
- **B** damages DNA and decreases the mutation rate
- **C** repairs DNA and increases the mutation rate
- **D** damages DNA and increases the mutation rate.

[Q3 2016 SCSA]

Questions 4 and 5 relate to the information that follows. A biologist measured the amount of genetic diversity in five populations of the Australian platypus. The amount of genetic diversity in each population is indicated by the diversity index. Values of the diversity index range from 0 (no diversity) to 1 (maximum diversity).

TABLE 4.7 Genetic diversity in five populations of the Australian platypus

POPULATION	DIVERSITY INDEX
Central Victoria	0.597
Northwestern Tasmania	0.606
King Island	0.032
Kangaroo Island (wild)	0.419
Kangaroo Island (sanctuary)	0.431

4 The mean value of the diversity index in the five platypus populations is:

A 0.346
B 0.417
C 0.236
D 0.504.

[Q22 2016 SCSA]

5 On the basis of the information in the table, which of the following platypus populations is at the greatest risk of extinction due to genetic factors?

A Kangaroo Island (wild)
B Kangaroo Island (sanctuary)
C Northwestern Tasmania
D King Island

[Q23 2016 SCSA]

6 Explain the role of fertilisation in sexual reproduction. (4 marks)

[Q35b 2019 SCSA]

7 Outline how crossing over creates genotypic variation. (2 marks)

[Q35c(i) 2019 SCSA]

8 Describe the effect that UV light has on DNA structure. (4 marks)

[Q35d 2019 SCSA]

9 Explain why mutation is the ultimate source of genetic variation. (4 marks)

[Q35e 2019 SCSA]

10 A study has shown that barn swallows living in an area contaminated by nuclear radiation have a higher incidence of abnormalities compared with those in uncontaminated areas. Provide a plausible explanation for the higher incidence of abnormalities in the barn swallows that live in the contaminated area. (4 marks)

[Q31e 2017 SCSA}

11 Describe the process of meiosis and explain how this process produces genetic variation. (10 marks)

[Q36b 2016 SCSA]

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5

GENETICS

CHAPTER 5 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 How did Mendel work out the inheritance of dominant and recessive traits without knowledge of genes and their different forms?
2 How are critical thinking skills applied in genetics?
3 Can you predict the mode of inheritance of a disease by examining a pedigree and using a systematic method?

SCIENCE UNDERSTANDING

» frequencies of genotypes and phenotypes of offspring are determined by patterns of inheritance, including dominance, autosomal and sex-linked alleles, multiple alleles and polygenes

SCIENCE INQUIRY SKILLS

» select, construct and use appropriate representations, including models of DNA replication, transcription and translation, Punnett squares and allele frequencies in gene pools, to communicate conceptual understanding, solve problems and make predictions

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

Getty Images/Max shen

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5.1 GENETICS INTRODUCTION

Have you noticed your hairline directly above your forehead? Does the hair take a V shape around the centre of the hairline, known as a 'widow's peak'? Is a widow's peak present or absent? The presence of a widow's peak is **dominant** over the absence (straight hair-line). If someone you know has a widow's peak, then one of their biological parents will definitely have this characteristic too. How do we know this for certain? Firstly, we can thank the father of **genetics**, Gregor Mendel, for studying **traits** in the 1800s and coming up with the basic principles of **inheritance**, and then thank all the scientists since who have further added to this knowledge.

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FIGURE 5.1 a Presence and **b** absence of a widow's peak on the hairline

To study patterns of inheritance, we will turn back the clock to examine the innovative experiments and observations of the Austrian monk, Gregor Mendel. Inheritance is the passing of traits from parents to offspring. Sexually reproducing organisms have two copies of almost every **gene**, one copy from the female parent and one copy from the male parent. The way genes are inherited can be studied, because there are patterns that emerge. Although offspring receive a combination of genetic material from two parents, certain genes will dominate in the expression of some traits. During this chapter on genetics, the principles of **heredity** and inherited variation will be investigated.

Getty Images/Time Life Pictures

FIGURE 5.2 Mendel discovered the key principles of inheritance in his pea garden.

The principles of heredity

The principles of heredity (inheritance of traits from one generation to the next) and patterns of inheritance were first established by Gregor Mendel (1822–84) in the 19th century. In about 1856, Mendel carried out a number of breeding experiments on pea plants in the garden at his monastery. The conclusions he drew from his studies form the foundations on which the study of heredity is built.

Mendel's peas and the inheritance principles

In the early stages of his work, Mendel studied the inheritance of seven pairs of contrasting characteristics in pea plants (Figure 5.3). These included variations such as yellow or green pea pods, round or wrinkled seeds, and tall-stemmed or dwarf-stemmed plants. Pea plants were ideal for his work: the characteristics ('variables') had no intermediate forms, pea plants self-pollinated and therefore self-fertilised, and the characteristics that he studied were largely unaffected by environmental factors.

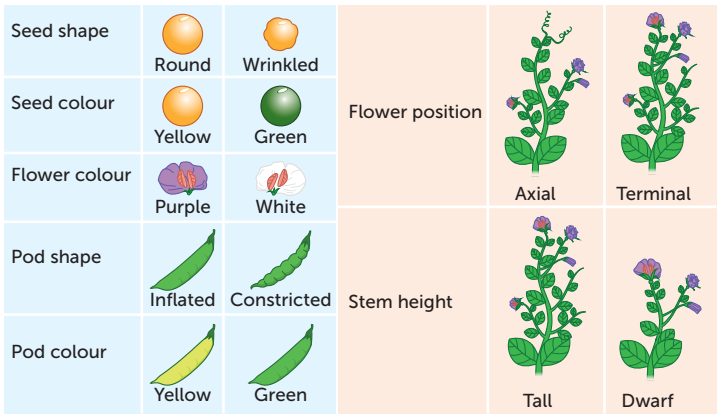

FIGURE 5.3 The pairs of characteristics of pea plants that Mendel studied

To understand Mendel's principles, some genetic vocabulary is required.

Genetics vocabulary

Allele

- A gene is the stored set of instructions for a protein, found on a specific locus (position) on a chromosome.
- **Alleles** are different forms of a gene.
- Pairs of alleles are found on a set of maternal and paternal homologous chromosomes.
- A set of alleles (one from each parent) is called a **genotype**.

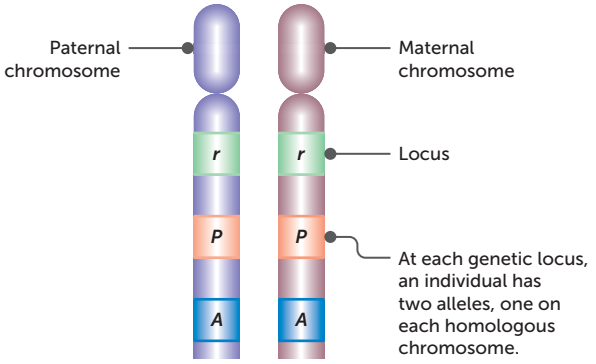

FIGURE 5.4 Pairs of alleles on homologous chromosomes

Dominant allele

- A dominant allele is always expressed in the **phenotype**.
- A dominant allele will mask a **recessive** allele.
- A dominant allele has the same effect on the phenotype whether it is paired with another dominant allele or a recessive one.
- A capital letter represents a dominant allele.

Recessive allele

- A recessive allele is only expressed in the phenotype when present with the same allele (**homozygous**) e.g. *ww*.
- A recessive allele is masked by a dominant allele.
- A lower-case letter represents a recessive allele.

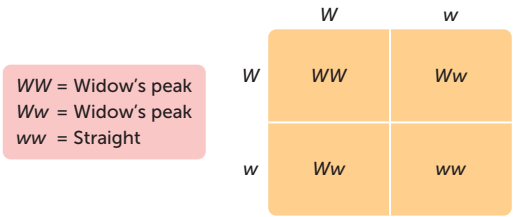

FIGURE 5.5 Dominant and recessive alleles

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Pure breed

- A pair of alleles in a pure breed are identical (it is homozygous).
- The set of alleles in a pure breed are either both dominant or both recessive for a specific trait.
- A pure breed for widow's peak can be either *WW* for widow's peak or *ww* for straight hairline.
- Pure breeds are used when studying inheritance and can be identified by a **test cross**.

Homozygous

- Homozygous means possessing two identical alleles of a gene.
- *AA* is a homozygous dominant genotype.
- *aa* is a homozygous recessive genotype.

Heterozygous

- **Heterozygous** means possessing two different alleles of a gene.
- *Aa* is a heterozygous genotype.
- The dominant allele will be expressed.

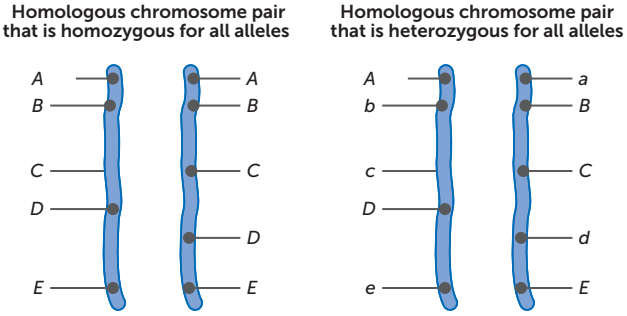

FIGURE 5.6 Homozygous and heterozygous alleles

Autosomal trait

- An **autosomal trait** is inherited on an autosome – a chromosome that is not a sex chromosome.
- A gene on an autosome is called autosomal.

Sex-linked trait

- A **sex-linked trait** is inherited on a sex chromosome (X or Y). A gene on a sex chromosome is called **sex-linked**.
- Note: the Y chromosome is short and contains relatively few genes. Most of them code for sex-related traits. X chromosomes are longer and contain more genes. They carry genes for sexual development as well as for certain other traits.

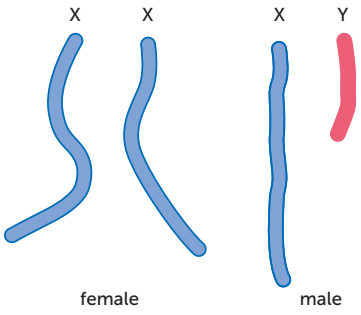

FIGURE 5.7 Sex chromosomes

Punnett square

- A **Punnett square** is a table that displays all the possible offspring genotypes (given the parental alleles) that can be produced at fertilisation. You can determine the likelihood of producing a child with a particular trait using a Punnett square. Assuming the genotypes of the parents for a trait are known using a Punnett square allows you to work out the potential genotypes of their offspring, as well as to determine the likelihood of a particular offspring having the trait.

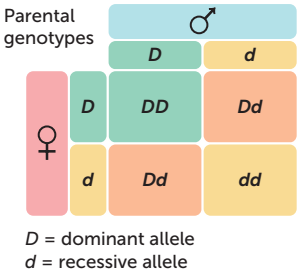

FIGURE 5.8 A Punnett square

Mendel's experiments

In one of Mendel's experiments, he took a **pure-breeding** tall pea plant and crossed it with a pure-breeding short pea plant. Pure-breeding plants are ones that, when crossed among themselves, always give rise to offspring that are like the parents. The way Mendel crossed the plants was to take pollen grains containing sperm cells from the anthers of one plant and dust them onto the stigma of another plant, having first removed the anthers of this second plant to ensure that it could not self-pollinate (Figure 5.9). This is referred to as hand pollination.

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Alamy/Edward Parker

FIGURE 5.9 Removing anthers to prevent self-pollination

Science Source/Nigel Cattlin

FIGURE 5.10 Tall and short pea plants: two different phenotypes for height

Mendel collected the seeds that resulted from the crosses between the tall pea plants and the short ones, and sowed these. He found that the seeds, once they had germinated and grown into adult plants, always developed into tall offspring. In these crosses, we refer to the original pure-breeding parent plants as the **parental generation (P)**. The offspring belong to what we call the **first filial generation (F_1)**. The term 'filial' means son or daughter.

Mendel then took the tall F_1 plants and self-pollinated each of them, this time taking precautions to prevent them from being pollinated by any other kind of pollen. The resulting seeds were sown and the offspring, belonging to the **second filial generation (F_2)**, were examined. Mendel found that some of these F_2 plants were tall and some were short. Overall, he counted 1064 plants. Of these, 787 (74%) were tall and 277 (26%) were short. It seemed as though approximately three-quarters of the F_2 generation were tall and one-quarter were short. In other words, the ratio of tall to short plants was approximately 3:1.

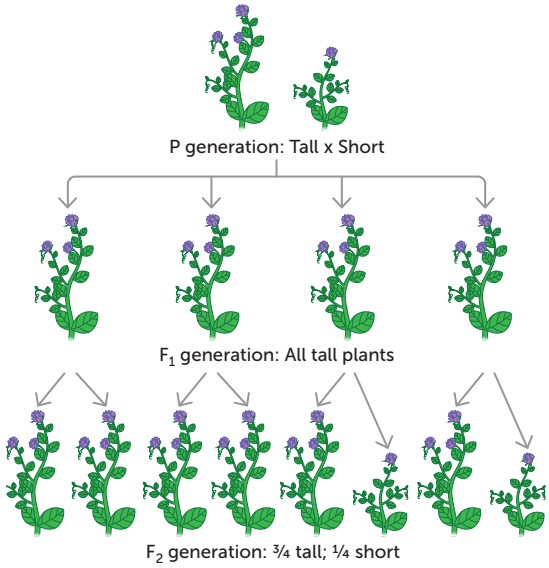


FIGURE 5.11 An example of Mendel's crosses: tall vs short pea plants

Mendel's conclusions

The first striking fact to notice in Mendel's results is that in the F_1 and F_2 generations there are no medium-sized plants; that is, there are no plants that are intermediate between the tall and the short parents. From this, we conclude that inheritance of this characteristic is not the result of the two parents' features blending together in the offspring. Rather, definite factors, which may or may not show themselves in the outward appearance of the organism, pass from parents to offspring. That such factors exist is borne out by the observation that, despite its absence in the F_1 generation, the short form reappears in the F_2 generation.

The second conclusion is drawn from the observation that there were no short plants in the F_1 generation, despite the fact that one of the parent plants was short. Short plants reappeared, however, in the F_2 generation. From this we can conclude that, although the F_1 plants are tall, they must receive a factor for shortness from their short parent, which remains 'hidden' in the F_1 plants and does not reveal its presence until the F_2 generation.

A third conclusion is that the factor for shortness, which fails to show itself in the F_1 generation, must be masked in some way by the factor for tallness. Only in the absence of this factor will the factor for shortness show itself in the outward appearance of the plant. In other words, the factor for tallness is dominant over the factor for shortness. Shortness is described as recessive.

Each generation can be predicted if the parent genotypes are known. The predictions of the offspring genotypes and phenotypes can be represented by Punnett squares.

Key concept

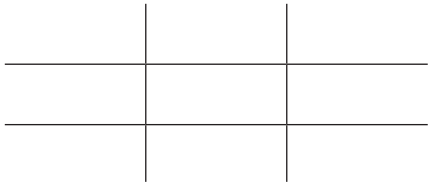
A phenotype describes the observable characteristics of an individual. Mendel was able to study the principles of genetics through careful observation of the phenotypes of pea plants and using selective breeding techniques. He found dominant traits masked recessive traits.

Inheritance principles: Punnett squares

A Punnett square is a diagram that shows all possible combinations of alleles and, therefore, all the possible genotypes of the offspring.

Reginald Punnett developed the 'Punnett Square' to depict the number and variety of genetic combinations.

How to construct a Punnett square



1. Draw a 2 by 2 Punnett square. Include an extra row and column for the parent alleles.
2. Decide on an appropriate letter to denote the dominant and recessive alleles. The dominant allele should have a capital letter (and be written first if the genotype is heterozygous), and the recessive allele should have a lower-case letter. This should be visible in a key along with the parent genotype cross.
3. Add the maternal parent genotype to the header row and the paternal parent genotype to the first column (or vice versa).

   The results for Mendel's pure-breeding tall and short peas will be used as an exemplar. The alleles are separated, as they are in meiosis. The single alleles would be found in gametes without their pair, ready for fertilisation.

   Key:

   Alleles

   T = tall (dominant allele)

   t = short (recessive allele)
4. Write out the possible combinations of the genotypes of the offspring of this successive generation.

   The offspring represent the possible F_1 generation.

Cross

Parent cross = *TT* × *tt*

	T	*T*
t		
t		

	T	*T*
t	*Tt*	*Tt*
t	*Tt*	*Tt*

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5 After the Punnett square is complete, determine the genotype and phenotype ratios. The ratios can be displayed as whole numbers, fractions or percentages.

Genotype ratio:	4 *Tt*	OR	¼ = 1 *Tt* (simplify fractions)	OR	100% *Tt*
Phenotype ratio:	4 tall	OR	¼ = 1 tall	OR	100% tall

The F_2 generation can be represented in the same way.

Key:

Alleles

T = tall (dominant allele)

t = short (recessive allele)

Cross

Parent cross = *Tt* × *Tt*

	T	*t*
T	*TT*	*Tt*
t	*Tt*	*tt*

Punnett square calculator

Genotype ratio:	1*TT* : 2 *Tt* : 1*tt*	OR	¼ *TT* : ½ *Tt* : ¼ *tt*	OR	25% *TT* : 50% *Tt* : 25% *tt*
Phenotype ratio:	3 tall : 1 short	OR	¾ tall : ¼ short	OR	75% tall : 25% short

Always include a genotype and phenotype key and ratios (even when not instructed to).

Key concept

A Punnett square is a visual representation that can be used to study and predict patterns of genetic inheritance.

Question set 5.1

REMEMBERING

1 Define pure-breeding.
2 Distinguish between a locus, a gene and an allele.
3 Define genotype and phenotype.
4 Define the P, F_1 and F_2 generations.
5 Define heterozygous and homozygous and state one example of a genotype for each.

UNDERSTANDING

6 How many different genotypes are possible for the tall and short traits of Mendel's peas? List them and classify them according to whether they are homozygous or heterozygous and whether they result in a dominant or recessive phenotype.

ANALYSING

7 Determine the phenotype ratio for the offspring of a homozygous tall pea plant crossed with a heterozygous tall pea plant. Show your working out in a Punnett square.
8 Explain Mendel's reasoning behind his conclusion that the factors he studied in pea plant height excluded an intermediary form (there was no blending of traits; plants were either tall or short).

5.2 GENETICS TODAY

Instead of 'factors' we use the term 'gene'. As Mendel observed, the gene controlling height in the pea plant exists in two forms, which we now call alleles. One allele functions in a certain way and is responsible for producing a tall plant. The other influences development in such a way that, if two copies of that allele are present together, a short plant is produced. Therefore, there are two distinct variations.

We can apply what we know about genetics today to Mendel's results. In Figure 5.12 (page 130), the allele for tallness is represented by *T* and the allele for shortness by *t*. We shall assume that each parent plant (or, more strictly, each somatic cell of each parent plant) contains a pair of identical alleles: *TT* in the case of the tall parent, *tt* in the case of the short parent. When an organism contains

identical alleles like this, it is said to be homozygous. In making this statement, we are describing the genetic make-up of the parent plants for height. The genetic composition (set of alleles) of an organism is known as its genotype. In essence, the genotype describes the alleles that a cell or organism has at a particular gene locus for a particular trait. The way genes are expressed in the outward appearance of the organism is known as its phenotype. In the case of the parental generation of pea plants described earlier, plant height is the phenotype. Pea plants with the 'tall' phenotype have the genotype *TT* or *Tt*. and pea plants with the 'short' phenotype have the genotype *tt*.

The *T* allele is present in each of the gametes produced by the tall parent, and the *t* allele is present in each of the gametes produced by the short parent. Fertilisation brings the *T* and *t* alleles together, so that all the F_1 offspring have the genotype *Tt*. Phenotypically, they are all tall, because tall is dominant over short. When an organism contains two dissimilar alleles, it is said to be heterozygous. If the organism is heterozygous with respect to one particular gene, it is called a **monohybrid**. In this particular instance, the *T* allele expresses itself in the phenotype, and the expression of the *t* allele is masked by the expression of the *T* allele. A dominant phenotype is expressed whether it occurs in the homozygous or the heterozygous condition. However, a recessive trait is only expressed when in the homozygous condition.

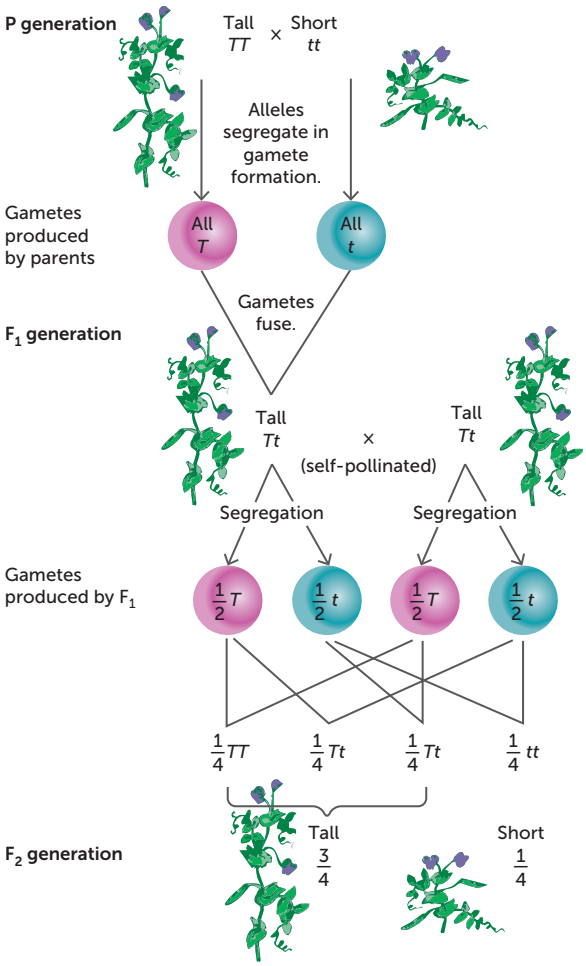

FIGURE 5.12 Summary of the cross between a pure-breeding tall pea plant and a pure-breeding short pea plant. This is an example of a monohybrid cross, because only one gene is involved on one locus.

Monohybrid cross: inheritance of a single autosomal gene

The 3:1 ratio Mendel predicted in the F_2 generation was based on observations of many different crosses using a variety of pea plant characteristics. Mendel could not explain why he observed this ratio because he had no knowledge of meiosis.

We can relate the behaviour of chromosomes at meiosis to the 3:1 ratio obtained. In meiosis, homologous chromosomes separate from each other, so that haploid gametes receive only one of each type of chromosome, instead of the two chromosomes present in diploid cells. In diploid cells, alleles occur in pairs, one of each pair being located on each of two homologous chromosomes (Figure 5.13). When homologous chromosomes separate in meiosis, the alleles are separated, so each gamete receives only one of a pair of alleles, just as they receive only one of a pair of homologous chromosomes (Figure 5.14).

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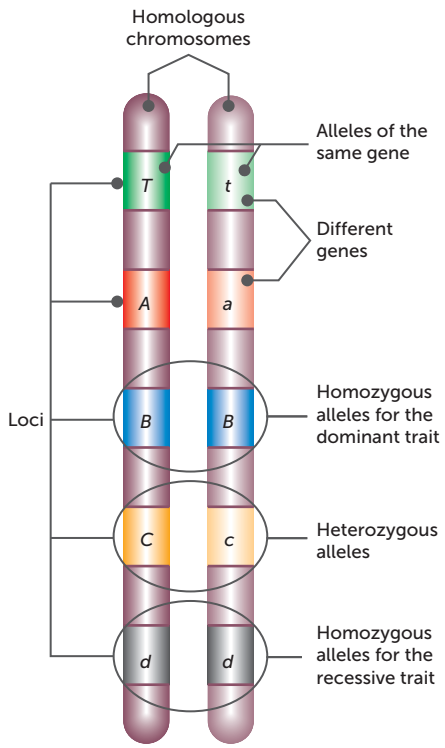

FIGURE 5.13 Combinations of alleles on homologous chromosomes

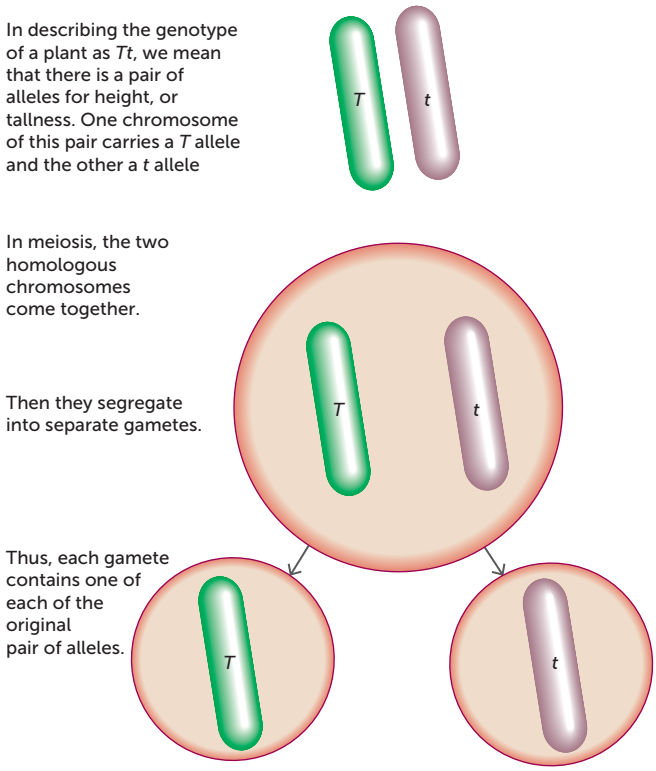

FIGURE 5.14 The segregation of alleles in inheritance corresponds to the segregation of homologous chromosomes in meiosis.

Key concept

Inheritance of alleles of a single autosomal gene can be analysed using a monohybrid cross. If the P generation is pure-breeding, the proportion of dominant to recessive alleles in the F_2 generation is typically 3:1.

A **monohybrid cross** involves fusion of gametes from two monohybrids (parents with genotypes consisting of one dominant and one recessive allele) that differ in only one characteristic. Only one gene is investigated. Recall that a monohybrid is an organism that is heterozygous with respect to a single gene. Monohybrids are the offspring from a cross between parents who are both homozygous but have different alleles from each other. Mendel performed crosses with pure-breeding pea plants, which produced monohybrids heterozygous for the gene of interest. This was the F_1 generation. When he crossed two organisms of the F_1 generation, he was crossing two monohybrids. The offspring of the monohybrid cross, known as the F_2 generation, gave rise to a 3:1 ratio of the dominant and recessive phenotypes. Mendel's cross between the offspring of the tall and short pea plants is an example of a monohybrid cross, using a parental generation with the genotypes (phenotypes in brackets) of *TT* (tall) and *tt* (short), and obtaining an F_1 generation of *Tt* (tall). The segregation of the alleles into the gametes can be explained in terms of meiosis, as illustrated in Figure 5.15.

When a monohybrid cross is presented to you in a question, a Punnett square can be used to predict the genotypes and phenotypes of the offspring, and in some cases to infer the genotypes and phenotypes of the parents. This technique can help plant or animal breeders develop varieties

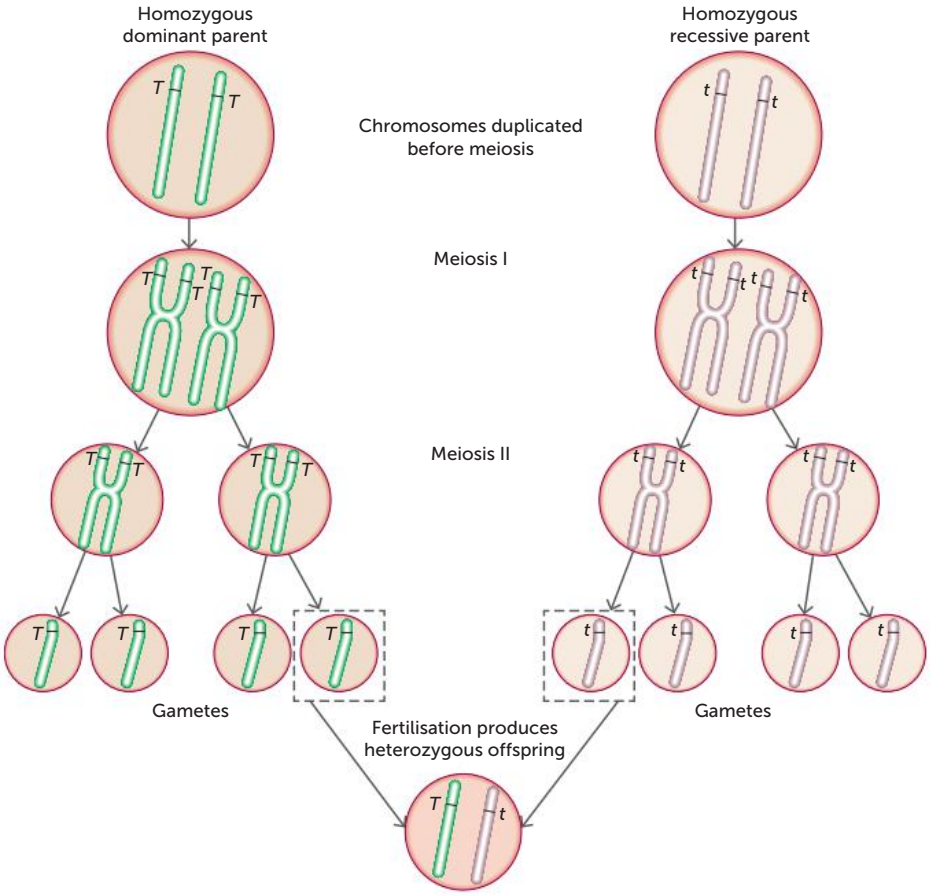

FIGURE 5.15 The segregation of chromosomes in a monohybrid cross: two homozygous parents with different phenotypes can only produce heterozygous offspring.

that have desirable traits. To solve genetics problems, you will need to apply your understanding and use **critical thinking**. Critical thinking involves reasoning with information to arrive at the best possible solution. It involves using your knowledge and understanding in a systematic way to solve problems and provide structured reasoning for your conclusions.

Worked example 5.1

Solving monohybrid cross questions

A pea plant that is heterozygous and has green pea pods is crossed with a pure-breeding pea plant for yellow pods. Predict the proportion of offspring that will produce green pea pods.

Sample marking allocation

- **a** Key (assign the alleles and state the cross). (1 mark)
- **b** Draw the Punnett square and enter the female and male gametes. (1 mark)
- **c** Determine the genotypes of all the possible offspring. (2 marks)
- **d** Determine the proportions of the genotypes and phenotypes of the offspring. (2 marks)

STEP	STRUCTURED REASONING	WORKING OUT	MARK ALLOCATION
a	If the green pea pod plant is heterozygous but still green, then the green allele is being expressed and is therefore the dominant allele. The dominant allele is represented by a capital letter. If the alleles are not defined, you will need to allocate an appropriate letter after you deduce which trait is dominant. The first parent genotype is *Gg*. The other parent is pure-breeding and therefore homozygous recessive. The recessive allele is represented by a lower-case letter. The second parent genotype is *gg*.	**Key:** Alleles *G* = green pod *g* = yellow pod Cross Parent cross = *Gg* × *gg*	1 mark
b	Due to meiosis and random segregation of chromosomes, each allele is separated, representative of the haploid gamete in which it resides. Place the parent alleles in separate columns and rows.	<table><tr><td></td><td>*G*</td><td>*g*</td></tr><tr><td>*g*</td><td></td><td></td></tr><tr><td>*g*</td><td></td><td></td></tr></table>	1 mark
c	The next step simulates fusion of the male and female gametes, giving all the possible allele combinations. Complete the Punnett square by pairing up the alleles to make diploid offspring.	<table><tr><td></td><td>*G*</td><td>*g*</td></tr><tr><td>*g*</td><td>*Gg*</td><td>*gg*</td></tr><tr><td>*g*</td><td>*Gg*</td><td>*gg*</td></tr></table>	2 marks
d	Each genotype with at least one *G* allele must express the dominant phenotype. The Punnett square shows that two of the four squares for the offspring have at least one *G* allele. Only the *gg* genotype will express the recessive phenotype. Whole number ratios must be simplified or represented as percentages or fractions.	Genotype ratio 2 *Gg* : 2 *gg* = 1 *Gg* : 1 *gg* OR 50% Gg : 50% *gg* Phenotype ratio 2 green : 2 yellow pods = 1 green : 1 yellow pod OR 50% green pods : 50% yellow pods	2 marks

Try these yourself

Using the alleles *P* and *p*, draw Punnett squares to show the ratio of purple to white plants among the offspring from the following crosses. Be careful you do not mix up your capital and lower-case letters.

1. A pure-breeding purple plant with a pure-breeding white plant.
2. A cross between two pea plants of the F_1 generation from the parental cross in Question 1.

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Question set 5.2

REMEMBERING

1 Distinguish between dominant and recessive alleles in terms of expression.
2 Define monohybrid cross.
3 State the purpose of a Punnett square.

UNDERSTANDING

4 Explain why the same genotype can result in a number of different phenotypes.
5 Complete the table of results of Mendel's crosses (Table 5.1) by simplifying F_2 ratios. The first two have been done for you as exemplars.

TABLE 5.1 Phenotype ratios

PURE-BREEDING PARENTAL PHENOTYPES	F_1 PHENOTYPES	F_2 PHENOTYPES	F_2 RATIO	F_2 RATIO ROUNDED TO THE NEAREST WHOLE NUMBER
Tall plants × short plants	All tall	787 tall, 277 short	2.84:1	3:1
Purple flowers × white flowers	All purple	705 purple, 224 white	3.15:1	3:1
Green pods × yellow pods	All green	428 green, 152 yellow		
Yellow peas × green peas	All yellow	6022 yellow, 2001 green		
Round peas × wrinkled peas	All round	5474 round, 1850 wrinkled		

6 Write a conclusion based on the table of results in Question 5.

ANALYSING

7 Describe the relationship between the process and products of meiosis and Mendel's 3:1 ratio in monohybrid crosses.

CREATING

8 Construct Punnett squares, determine the genotype and phenotype ratios, and provide an answer for the following questions.
   a The presence of freckles is a dominant characteristic. An unborn child's mother has no freckles and its father is heterozygous for freckles. What is the probability that this child will have freckles?
   b In a variety of garden peas, the allele for tall plants (*T*) is dominant over the allele for short plants (*t*). A cross between a tall plant and a short plant resulted in 50% of the offspring being short. What were the genotypes of the parents?

5.3 THE TEST CROSS

If an organism's genotype is unknown, and it is displaying a dominant phenotype, it is possible to predict the genotype by performing a test cross. This technique involves crossing the individual whose genotype is unknown but that has a dominant phenotype (it could be homozygous dominant or heterozygous dominant) with an organism that is homozygous recessive at the locus in question. This is called a test cross. The ratio of phenotypes in the offspring reveals the unknown genotype. We can illustrate this by reference to an animal that is used in many genetic experiments – the fruit fly, *Drosophila melanogaster*.

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D. melanogaster has a large number of variants or forms. Most individuals have long wings, but some have small or 'vestigial' wings (Figure 5.16). The long-winged condition (*V*) is dominant to vestigial wing (*v*). Accordingly, if a pure-breeding long-winged fly (*VV*) is mated with a vestigial-winged fly (*vv*), the F_1 individuals are all heterozygous at this locus (*Vv*) and have long wings. If two of these F_1 flies mate with each other, a mixture of long-winged and vestigial-winged flies are produced in a ratio of approximately 3:1 (Figure 5.17).

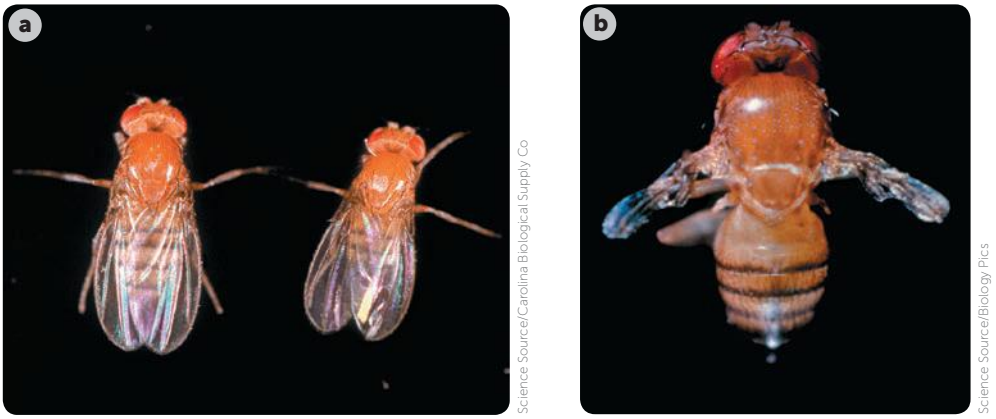

FIGURE 5.16 The fruit fly *D. melanogaster* may have **a** long wings or **b** vestigial wings.

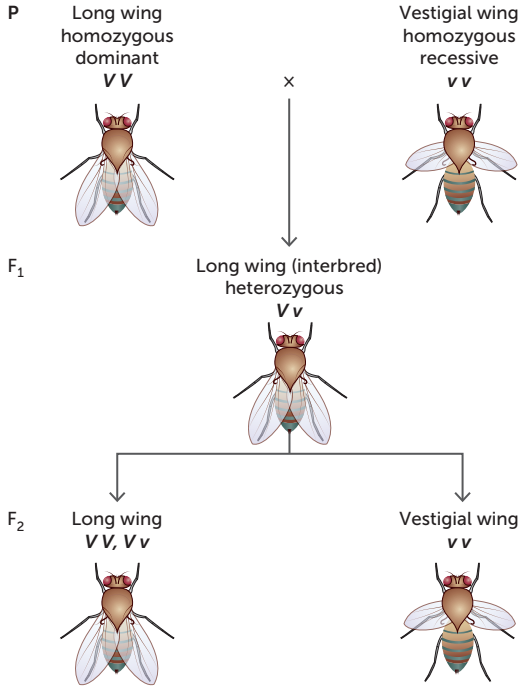

FIGURE 5.17 Monohybrid cross showing inheritance of wing length in fruit flies

This is expected for a monohybrid cross. But how can we decide whether a given F_2 long-winged fly is homozygous dominant (*VV*) or heterozygous (*Vv*)? The simplest way to solve this problem is to cross it with a vestigial-winged fly. We know that a vestigial-winged fly must be *vv* (homozygous recessive); it cannot be anything else. If the long-winged fly whose genotype we wish to determine is *VV*, then crossing it with a vestigial-winged fly will give nothing but long-winged flies. If, however, the unknown fly has the genotype *Vv*, then the cross will give a mixture of long- and vestigial-winged flies in approximately equal numbers. This is summarised in Figure 5.18 (page 136).

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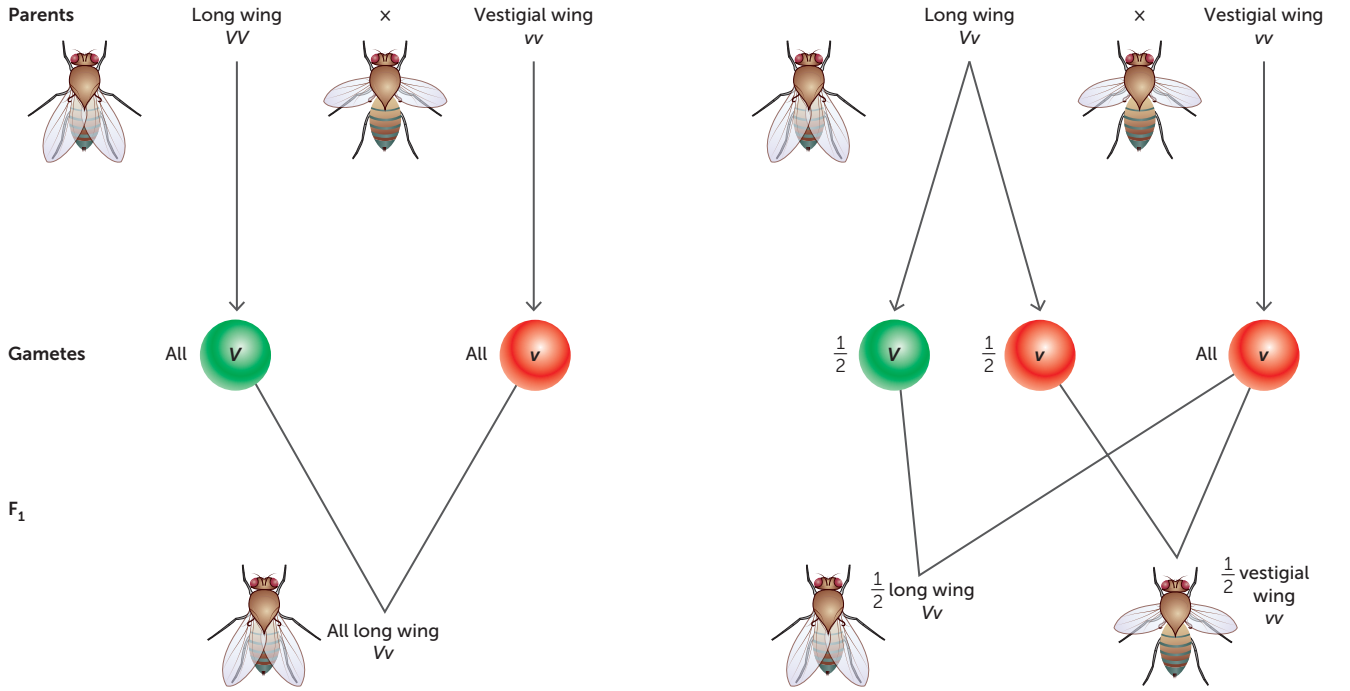

FIGURE 5.18 A test cross to determine whether a fruit fly is homozygous or heterozygous for long wings

Test crosses using homozygous recessive individuals are a routine method of establishing an organism's genotype.

In guinea pigs, black fur colour is dominant over white. A test cross could be performed to find out the genotype of a black pet guinea pig of unknown genotype. If the owner wanted to be certain their pet was homozygous dominant black, for breeding purposes, they could breed the pet with a guinea pig who was homozygous recessive white.

FIGURE 5.19 A test cross can help determine the genotype of a pet guinea pig.

When an organism displaying a dominant phenotype is crossed with an organism that is homozygous recessive for the condition, there are two possible outcomes. Either 100% or 50% of the offspring will present the dominant phenotype. Below are the two possible crosses, given the black phenotype could have arisen from the homozygous or heterozygous set of alleles. Compare the results.

PUNNETT SQUARE IF THE BLACK GUINEA PIG'S GENOTYPE IS *BB*	PUNNETT SQUARE IF THE BLACK GUINEA PIG'S GENOTYPE IS *Bb*
		B	*B* | *b*	*Bb*	*Bb* | *b*	*Bb*	*Bb*			*B*	*b* | *b*	*Bb*	*bb* | *b*	*Bb*	*bb*
Genotype ratio 100% *Bb* Phenotype ratio 100% black	Genotype ratio 50% *Bb* : 50% *bb* Phenotype ratio 50% black : 50% white

A conclusion about the genotype can be drawn after analysing the offspring phenotype ratios. If any offspring appear white, a breeder can be certain the unknown genotype was heterozygous (*Bb*). If the offspring are all black, the breeder can conclude the unknown genotype is homozygous (*BB*).

Question set 5.3

REMEMBERING

1. State the purpose of a test cross.
2. To find the genotype of an individual displaying a dominant phenotype, what type of genotype should it be crossed with?

UNDERSTANDING

3. Explain how a test cross helps a breeder determine the genotype of an organism.

CREATING

4. In the fruit fly, *D. melanogaster*, the ebony-body allele (*e*) is recessive to the normal yellow-body allele (*E*). Predict the genotype of a male fly with a yellow body by:
   - a. constructing the two possible Punnett squares for a test cross with an ebony body female fly
   - b. converting your predicted offspring into
     - i. percentages
     - ii. fractions.

5.4 MULTIPLE ALLELES FOR ONE GENE

Only two alleles account for each of the pea plant traits that have been discussed so far. For most traits, there are more than two alleles for a gene. This phenomenon is known as having **multiple alleles**. In any one individual, of course, only two alleles are normally present. A multiple allele system is present when three or more alleles of a gene exist among the members of a population. An example of this is the ABO blood group system in humans, where there are three alleles possible for one gene. The phenotype expressed by allele I^A, which is codominant with I^B, produces molecular markers on red blood cells. We discuss codominance in more detail later in this chapter (see page 148). The phenotype expressed by the third allele *i* is recessive to both I^A and I^B and produces no marker (O). Table 5.2 summarises the genotypes and the resulting phenotype (blood groups).

TABLE 5.2 Genotypes and phenotypes for human blood groups

PHENOTYPE	GENOTYPE
Blood type A	I^AI^A or I^Ai
Blood type B	I^BI^B or I^Bi
Blood type AB	I^AI^B
Blood type O	*ii*

The fact that there are more than two alleles responsible for determining blood group makes no difference to their transmission, which takes place in a normal Mendelian fashion. Thus, a child whose parents are both blood group O must be blood group O. Consider the offspring of a man who is blood group AB and a woman who is heterozygous blood group B. A Punnett square can still be used to determine the possible genotype and phenotype ratios.

Key:

Alleles

I^A = Group A

I^B = Group B

i = Group 0

Cross

Parent cross = $I^AI^B \times I^Bi$

	I^A	I^B
I^B	I^AI^B	I^BI^B
i	I^Ai	I^Bi

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Genotype ratios: 1 I^AI^B : 1 I^BI^B : 1 I^Ai : 1 I^Bi

Phenotypes ratios: 1 blood group AB : 2 blood group B : 1 blood group A

In the population, there are four possible phenotypes, with no one showing variation that is in between each blood group. This produces **discontinuous variation**, because only one set of alleles for one gene determines the phenotype. Discontinuous variation is a set of discrete phenotypic categories controlled by a single gene and its set of alleles. A human is either blood group A or B or AB or O, because these are the discrete categories coded for by one set of alleles for one gene. When characteristics are controlled by a single gene, the phenotypes fall into separate categories.

Discrete variation in a population can be represented on a bar graph.

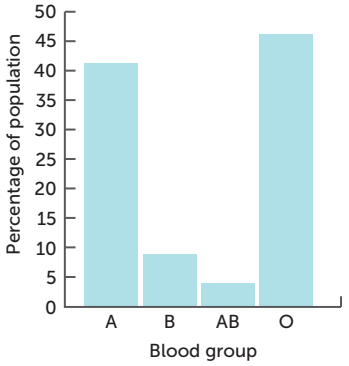

FIGURE 5.20 Blood groups in a population, showing discontinuous variation

Question set 5.4

REMEMBERING

1. State the possible genotypes for blood group A and blood group O.
2. Define discontinuous variation.

UNDERSTANDING

3. Draw a Punnett square to show that a person with blood group AB cannot have children with blood group O.
4. Provide an explanation for why a bar graph is used to display discontinuous variation.

ANALYSING

5. The baby ID bracelets of three newborns in the maternity ward of a hospital have been misplaced. Testing reveals the blood type of the babies to be one each of AB, A and O. For a couple where the mother is homozygous type A and the father is type O, predict which of the three babies is theirs. Ensure you include Punnett squares and ratios in your working out.

5.5 THE DIHYBRID CROSS

Dihybrid crosses involve two genes with two different alleles for each gene. In Mendel's genetic experiments on pea plants, he studied the inheritance of two traits at the same time. He also studied how these traits were inherited through two generations, finding the ratios of the resulting offspring. One pair of traits that he studied at the same time in pea plants was height and flower colour. He took pure-breeding (homozygous for both traits) strains of pea plants and crossed them. One plant was tall and had purple flowers. The other plant was short and had white flowers. He found that the offspring of a cross between these parents always produced plants that were tall and purple-flowered. This was the F_1 generation.

Members of the F_1 generation were then self-pollinated. In the F_2 generation, there were four different phenotypes: tall plants with purple flowers; tall plants with white flowers; short plants with purple flowers; and short plants with white flowers. In other words, the offspring showed the two pairs of characteristics (tall, short; purple, white) combined in every possible way.

As before, Mendel counted the different types of plants and found 96 tall purple plants, 31 tall white plants, 34 short purple plants and 11 short white plants, giving a ratio of approximately 9:3:3:1. The results of the experiment are summarised in Figure 5.22. What conclusions can be drawn from these results? First, the observation that all the F_1 plants were tall with purple flowers confirms that tall is dominant to short, and purple flower is dominant to white flower. This is as expected from the results of the monohybrid crosses.

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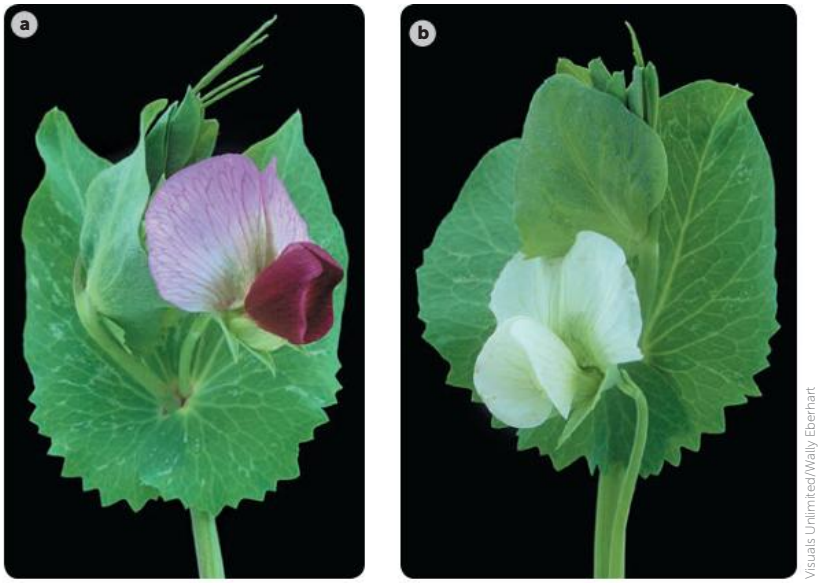

FIGURE 5.21 Pea plant flowers may be **a** purple or **b** white.

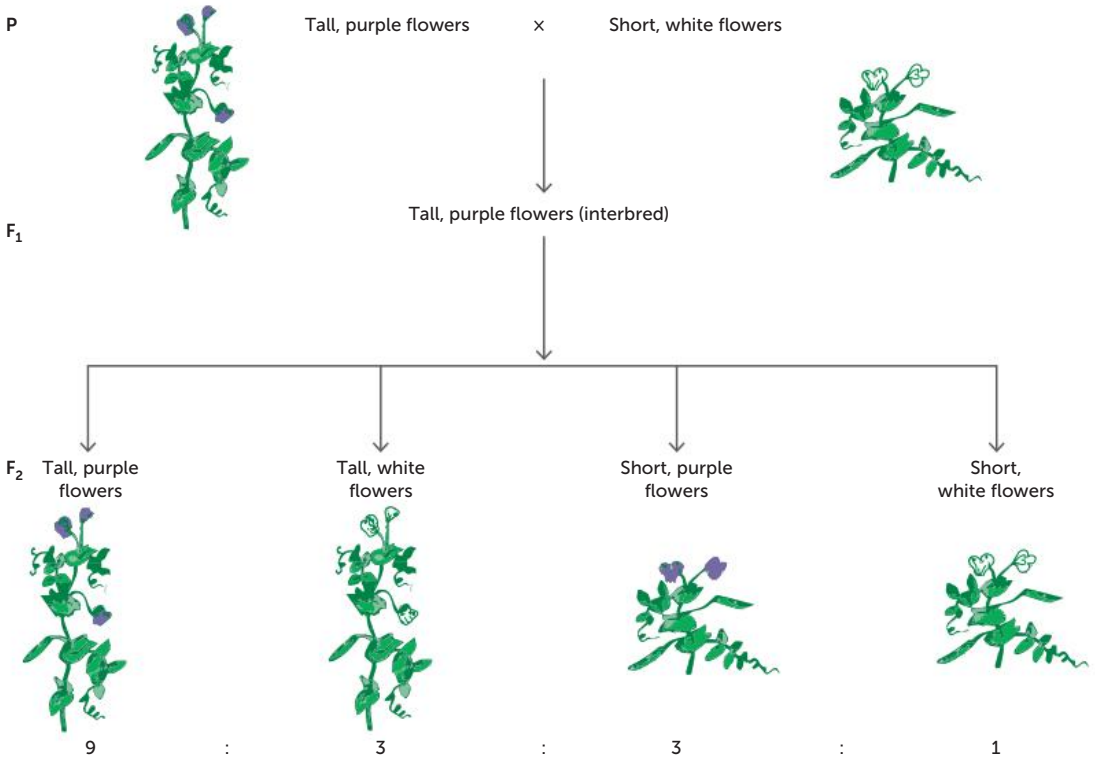

FIGURE 5.22 Summary of Mendel's dihybrid cross

Figure 5.23 (page 140) shows how the alleles were transmitted in this dihybrid cross. *T* represents the allele for tallness, *t* for shortness, *P* for purple flowers and *p* for white flowers. Mendel always started his experiments with pure-breeding plants, so the parent plants were homozygous for both genes. The genotype of the tall plant with the purple flowers was, therefore, *TTPP*, and that of the

short plant with white flowers was *ttpp*. From Mendel's earlier work, we know that the gametes produced by the parent plants were *TP* from the tall purple parent and *tp* from the short white parent. All the F_1 offspring, therefore, had the genotype *TtPp*, heterozygous for both genes.

The next step in the line of reasoning is crucial. Since all four possible combinations of characteristics showed up in the F_2 generation, we must conclude (as Mendel did) that the F_1 plants produced four kinds of gamete: *TP*, *Tp*, *tP* and *tp*. Figure 5.24 shows the different ways these gametes could combine, together with the genotypes of the F_2 offspring. To be tall, the genotype of the plant must contain at least one *T* allele; to be purple, it must contain at least one *P* allele. From the Punnett square, we can see that there are 16 possible combinations. Of these, 9 give tall purple plants, 3 tall white plants, 3 short purple plants and 1 short white plants. The observed 9:3:3:1 ratio can be accounted for if all the possible combinations occur with equal likelihood.

The basic structure of a dihybrid cross Punnett square is a 4 × 4 grid for possible offspring, with the four possible gamete allele combinations of one parent written across the top row and those of the other parent written down the left column. A key is drawn up for the alleles and the cross.

Key concept

Inheritance for two unlinked autosomal genes can be analysed with a dihybrid cross. If the P generation are pure-breeding with respect to all traits, the F_2 generation typically shows a ratio of 9:3:3:1.

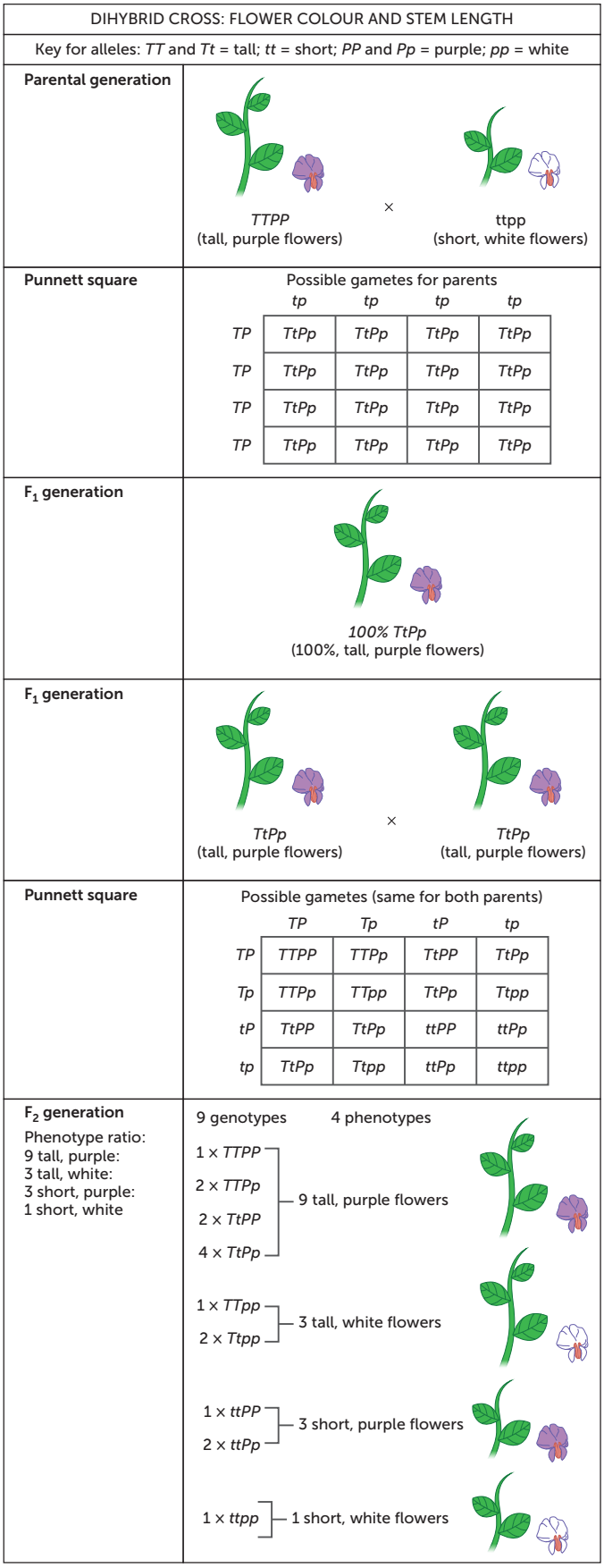

FIGURE 5.23 Dihybrid cross: F_1 and F_2 genotypes and phenotypes

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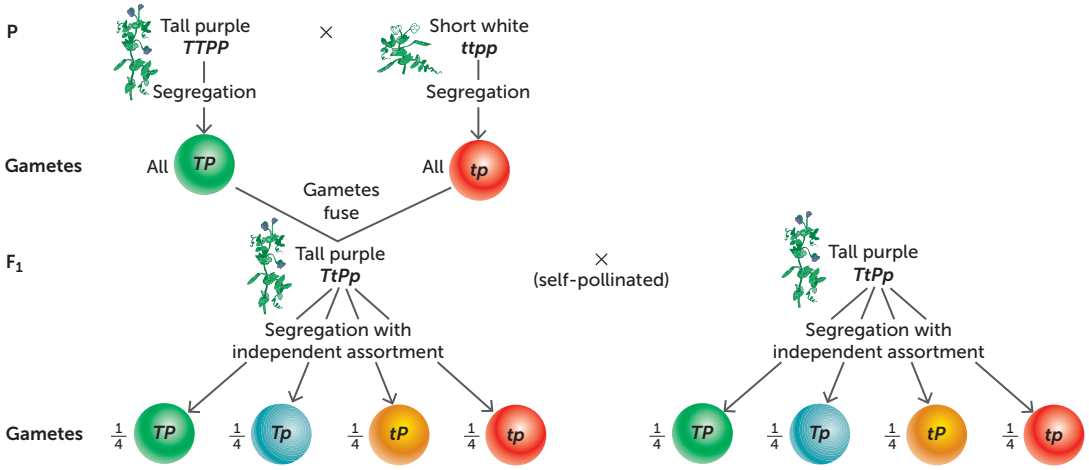

Punnett square to show fusion of F_1 gametes

		Male gametes			
		1/4 TP	1/4 Tp	1/4 tP	1/4 tp
Female gametes	1/4 TP	1/16 TTPP Tall purple	1/16 TTPp Tall purple	1/16 TtPP Tall purple	1/16 TtPp Tall purple
	1/4 Tp	1/16 TTPp Tall purple	1/16 TTpp Tall white	1/16 TtPp Tall purple	1/16 Ttpp Tall white
	1/4 tP	1/16 TtPP Tall purple	1/16 TtPp Tall purple	1/16 ttPP Short purple	1/16 ttPp Short purple
	1/4 tp	1/16 TtPp Tall purple	1/16 Ttpp Tall white	1/16 ttPp Short purple	1/16 ttpp Short white

F_2	9/16 Tall purple	3/16 Tall white	3/16 Short purple	1/16 Short white

FIGURE 5.24 Dihybrid cross of a pure-breeding tall purple-flowering pea plant with a pure-breeding short white-flowering pea plant: how to derive the possible combinations of alleles for each gamete.

Worked example 5.2

Solving a dihybrid cross

A pure-breeding fruit fly that has curly wings and red eyes is crossed with a pure-breeding fruit fly that has straight wings and purple eyes (Figure 5.25). Their offspring all have curly wings and red eyes. Show this in a Punnett square.

Two of the F_1 generation flies are crossed. If the alleles for wing shape and eye colour assort independently, predict the phenotypes of the F_2 generation and the proportions of each phenotype.

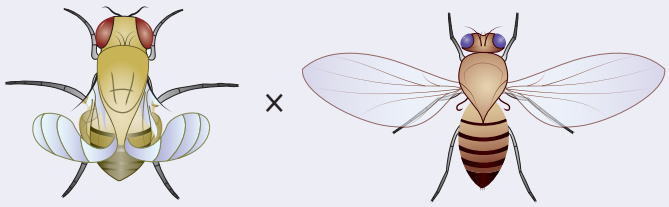

FIGURE 5.25 Pure-breeding fruit flies with curly wings and red eyes are crossed with pure-breeding fruit flies with straight wings and purple eyes.

Sample marking allocation

a Key (assign the alleles and state the cross). (1 mark)
b Draw the Punnett square and enter the female and male gametes. (1 mark)
c Determine the genotypes of all the possible offspring. (2 marks)
d Determine the proportions of the genotypes and phenotypes of the offspring. (2 marks)

STEP	STRUCTURED REASONING	WORKING OUT
a	The P generation flies are pure-breeding, so they are homozygous for wing shape (curly or straight) and eye colour (red or purple). Due to meiosis and random segregation of chromosomes and their alleles, each allele is separated from its pair, as are all the alleles in the haploid gamete it occurs in. Place the parent alleles in separate columns/rows. It follows that all the F_1 generation must be heterozygous with respect to both wing shape and eye colour. All the F_1 generation have curly wings and red eyes. This indicates that curly wings are dominant to straight wings and red eyes are dominant to purple eyes.	**Key:** Alleles *C* = curly wing *c* = straight wing *R* = red eyes *r* = purple eyes Cross Parent cross = *CCRR* × *ccrr* The different combinations of alleles are represented in the parent row and column. | | *CR* | *CR* | *CR* | *CR* | | *cr* | *CcRr* | *CcRr* | *CcRr* | *CcRr* | | *cr* | *CcRr* | *CcRr* | *CcRr* | *CcRr* | | *cr* | *CcRr* | *CcRr* | *CcRr* | *CcRr* | | *cr* | *CcRr* | *CcRr* | *CcRr* | *CcRr* |
b	The F_1 cross is between heterozygous fruit flies. The alleles for each trait (*Cc* and *Rr*) assort independently of one another, so four equally likely combinations of alleles are present in the gametes of the F_1 generation A Punnett square is used to determine the possible genotype and phenotype ratios of the F_2 generation.	The F_1 cross = *CcRr* × *CcRr*: | | *CR* | *Cr* | *cR* | *cr* | | *CR* | | | | | | *Cr* | | | | | | *cR* | | | | | | *cr* | | | | |
c	As with the monohybrid cross, each square is filled with the product of the combining female and male gametes. This simulates fusion of the male and female gametes, giving all the possible allele combinations. The 16 offspring represent the possible zygotes formed by different combinations of gametes.	The possible F_2 generation of offspring is filled in. | | *CR* | *Cr* | *cR* | *cr* | | *CR* | *CCRR* | *CCRr* | *CcRR* | *CcRr* | | *Cr* | *CCRr* | *CCrr* | *CcRr* | *Ccrr* | | *cR* | *CcRR* | *CcRr* | *ccRR* | *ccRr* | | *cr* | *CcRr* | *Ccrr* | *ccRr* | *ccrr* |

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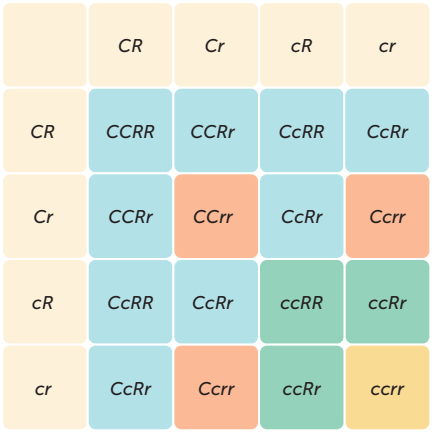
d	Nine of the 16 squares show offspring with at least one *C* and at least one *R* allele, and these offspring will have both dominant phenotypes, curly wings and red eyes. Three of the 16 squares show offspring with at least one *C* allele for curly wing shape and two *rr* alleles for purple eye colour. Three of the 16 squares show offspring with two *cc* alleles for straight wings and at least one *R* allele for red eyes. Just one of the 16 squares shows offspring with the genotype *ccrr* for both recessive phenotypes: straight wings and purple eyes. Therefore the phenotypic ratio is 9:3:3:1.	To determine the ratio of phenotypes, it is helpful to code the different categories. **Key:** Blue = curly wings and red eyes Orange = curly wings and purple eyes Green = straight wings and red eyes Yellow = straight wings and purple eyes  The phenotypic ratio is: 9 curly wings and red eyes : 3 curly wings and purple eyes : 3 straight wings and red eyes : 1 straight wings and purple eyes

Try these yourself

1. A pure-breeding fruit fly that has curly wings and long legs is crossed with a pure-breeding fruit fly that has straight wings and short legs. Their offspring all have curly wings and long legs. Two of the F_1 generation flies are crossed. If the alleles for wing shape and leg length assort independently, predict the phenotypes of the F_2 generation and the proportions of each phenotype.
2. A pure-breeding pea plant with yellow wrinkled peas was crossed with a pure-breeding pea plant bearing round green peas. All the F_1 offspring have round yellow peas. If two of the F_1 pea plants are crossed, predict the possible combinations of pea phenotypes with respect to shape and colour and the proportions in which they are likely to occur.

Explanation of dihybrid ratios

The observation that characteristics such as height and flower colour are inherited independently of one another is known as **independent assortment**. The explanation for this lies in the behaviour of the chromosomes at meiosis, which is just like the segregation of alleles that Mendel observed in his monohybrid crosses.

Independent assortment occurs when the genes concerned are carried on different chromosomes; for example, the alleles of the gene for flower colour are located on one pair of chromosomes and the alleles of the gene for height are located on another pair of chromosomes. In metaphase I of meiosis, homologous chromosomes line up side by side on the spindle prior to separating at anaphase I.

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The different pairs of homologous chromosomes act independently of one another: the way one pair of homologous chromosomes arranges itself on the spindle and subsequently separates has no effect whatsoever on the behaviour of any other pair of chromosomes.

The consequence of the independent behaviour of **non-homologous chromosomes** in meiosis is shown in Figure 5.26, which illustrates how the four different types of gametes (*TP*, *Tp*, *tP* and *tp*) can be formed from a plant that is heterozygous for height and flower colour (*TtPp*).

We can summarise the situation by saying that the alleles for height and flower colour segregate and assort independently because they are carried on separate chromosomes, which themselves segregate and assort independently in meiosis.

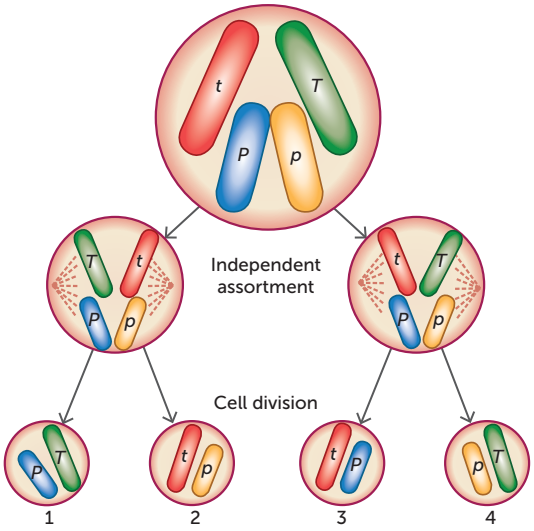

FIGURE 5.26 Meiosis provides the explanation for the independent assortment that Mendel observed in dihybrid crosses. The independent assortment of alleles in inheritance corresponds to the free assortment of chromosomes during meiosis.

Question set 5.5

REMEMBERING

1 State the expected phenotypic ratio for a dihybrid cross for an organism heterozygous for both genes.

2 Define non-homologous chromosomes.

UNDERSTANDING

3 Relate the term 'non-homologous chromosomes' to the 9:3:3:1 phenotypic ratio seen in the F_2 generation of a dihybrid cross.

ANALYSING

4 The genotypes of the offspring resulting from a dihybrid cross are shown in the Punnett square that follows.

Key:
Alleles
R = red flowers
r = yellow flowers
T = tall plant
t = dwarf plant

	RRTT	*RRTt*	*RrTT*	*RrTt*
	RRTt	*RRtt*	*RrTt*	*Rrtt*
	RrTT	*RrTt*	*rrTT*	*rrTt*
	RrTt	*Rrtt*	*rrTt*	*rrtt*

a Deduce the genotypes and phenotypes of the parents.

b Explain how the phenotypic ratio would differ depending on whether two genes are carried on homologous chromosomes or on two non-homologous chromosomes.

5 Two pure-breeding rabbits are mated: a doe with grey fur and black eyes is mated with a buck with white fur and red eyes. The litter contains only offspring with grey fur and black eyes. Assign the alleles and show the genotypes of the P and F_1 individuals. Draw a Punnett square to show a cross between individuals of the F_1 generation and predict the ratio of phenotypes with respect to fur and eye colour in the F_2 generation.

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Genetic crossing in Australian agriculture for high-yield, drought- and disease-resistant wheat varieties

CASE STUDY

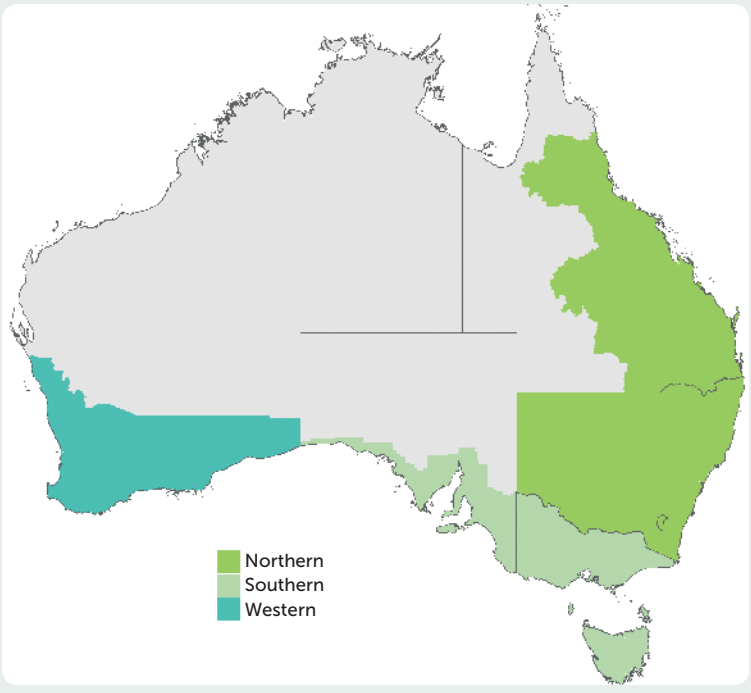
The Grains Research and Development Corporation (GRDC) is a statutory authority established to plan and invest in research, development and extension of the Australian grain industry. The grain industry contributed $12.8 billion to the total gross value of farm production in 2019, with wheat being the most valuable crop.

The image below shows the GRDC grain regions in Australia.

Drought, pests, frost, soil salinity and the disease wheat rust are ongoing issues for farmers of grains. These factors affect the financial performance of farmers and the export industry. WA is the largest exporter of wheat of the Australian states and territories, with markets in Asia and the Middle East, including Indonesia, Japan, South Korea, Malaysia and Sudan. Scientists and farmers are working together to develop grains that are high yield, and drought and disease resistant. Australian Grain Technologies (AGT) have started looking at a number of genes that have the potential to enable wheat to tolerate drought conditions.

Plant breeders have developed techniques for breeding crops with more desirable traits. In the past, crop improvements were made by selecting plants for breeding based on phenotypes and, through a system of trial and error over generations, obtaining seeds containing alleles for the desirable characteristics. Modern genetic analysis techniques enable scientists to be more precise and efficient in this endeavour. Phenotypes that are desirable in grains are salt tolerance, disease resistance, large grains, and drought resistance. If the right alleles can be identified, Australian farmers may continue harvesting grains, despite climate change.

The traits (phenotype characteristics) breeders can work with are affected by the plant's set of alleles. Alleles of a particular gene are variant forms of the same gene. In grain crops, the allele for a tall stem is dominant and the allele for a short stem is recessive. Alleles are represented by a single letter and are passed onto offspring in different combinations. If famers allow only tall plants to produce offspring, then all offspring will have the same tall phenotype, increasing yield.



Source: Grains Research & Development Corporation

FIGURE 5.27 GRDC grain regions in Australia

Currently, the GRDC funds research programs at the University of Western Australia, such as chickpea crop resilience.

Adapted from *The science of crossing and crops* - Grains Research and Development Corporation

Questions

1. List the environmental factors that affect grain production in WA.
2. Recall the relationship between genotype and phenotype.
3. Explain how a scientist may find out which allele is dominant or recessive in wheat plants.
4. Farmers choose specific wheat varieties to breed. Justify this decision.

Fairfax Photos/TAMARA VONINSKI

FIGURE 5.28 Wheat species growing in Beacon, WA – a very dry environment. Scientists are looking at various genes that have the potential to help wheat cope with drought conditions.

5.6 POLYGENIC INHERITANCE

'Poly' comes from the Greek word for many. **Polygenic inheritance** is the inheritance of more than one gene that affects the inheritance of a single characteristic. For one characteristic, two or more genes and therefore two or more sets of alleles contribute to a phenotype. A characteristic controlled by more than one gene is known as a polygenic characteristic. **Polygenes** are genes that have a small additive effect on a phenotype, and each gene consists of multiple alleles.

An example of polygenic inheritance is human height. Unlike Mendel's pea plants, which were either tall or short, humans have a range of heights, with a smooth gradation from one extreme to another. This can be seen when you line up for your school photographs each year. Whether it is a year or other group photograph, the students will vary in height. According to the Australian Bureau of Statistics' Health Survey of 2011–12, the average adult height in Australia was 161.8 cm for women and 175.6 cm for men. In the last 40 years, many genes contributing to height have been identified by their association with short stature or overgrowth. Additional genes associated with human height have been identified recently through studies of the human genome, and the total now exceeds 200 genes.

The condition of showing a range of phenotypes is called **continuous variation**. Traits that show continuous variation are controlled by two or more genes (polygenes). The greater the number of genes and the greater the influence of environmental factors (e.g. nutrition and the standard of medical care), the greater the expected distribution of all phenotypes for height, and the smaller the difference between any two individuals ordered along the spectrum of variation.

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Continuous variation in a group of individuals can be shown using a histogram (Figure 5.29). Discontinuous variation occurs when only one gene is involved. It results in a small number of discrete phenotypes, such as pea plants with either purple flowers or white flowers, but no colours other than these.

a

	ABC	*ABc*	*AbC*	*aBC*	*Abc*	*aBc*	*abC*	*abc*
ABC	6	5	5	5	4	4	4	3
ABc	5	4	4	4	3	3	3	2
AbC	5	4	4	4	3	3	3	2
aBC	5	4	4	4	3	3	3	2
Abc	4	3	3	3	2	2	2	1
aBc	4	3	3	3	2	2	2	1
abC	4	3	3	3	2	2	2	1
abc	3	2	2	2	1	1	1	0

b

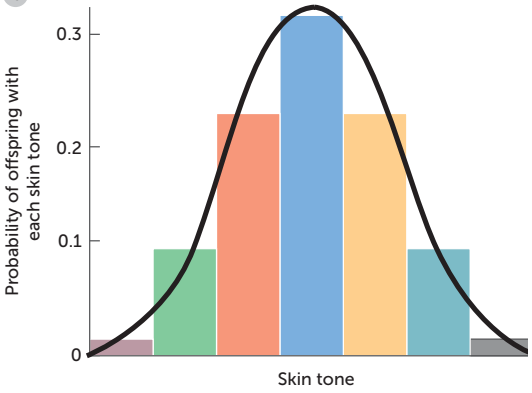

FIGURE 5.29 Continuous variation in skin tone is due to the additive effects of at least three melanin-producing genes, represented here as *A*, *B* and *C*. The alleles *a*, *b* and *c* do not produce melanin. **a** Punnett square of genotypes and **b** histogram showing the probability of each phenotype in the offspring of *AaBbCc* parents.

Key concept

An organism's phenotype is influenced by the alleles in its genotype. The inheritance of alleles can be either single-gene inheritance (two or more alleles at a single locus) or polygenic inheritance (multiple alleles at multiple loci, each contributing to the phenotype).

TABLE 5.3 Comparing continuous and discontinuous variation

FACTOR	CONTINUOUS VARIATION	DISCONTINUOUS VARIATION
Type of inheritance	Polygenic inheritance (multiple alleles for each gene and several genes)	Single-gene inheritance (but multiple alleles)
Cause	Genetic and environmental factors	Genetic factors
Description	Variation that shows gradual changes from one trait to another. Differences are slight, along a continuum.	Variation that shows clear and discrete changes between traits. There is no intermediate form.
Type of graphical representation	Histogram (a line graph can be superimposed to show continuous data)	Bar graph
Examples	Height, weight, skin colour	Ability or inability to tongue roll; attached or detached ear lobes; blood groups A, B, AB and O

Question set 5.6

REMEMBERING

1. Define polygenic inheritance.
2. Describe independent assortment.
3. Distinguish between multiple alleles and polygenes.

UNDERSTANDING

4. Explain why some phenotypes, such as height, can show continuous variation, yet others show discontinuous variation.

APPLYING

5. Provide a human and a non-human example of polygenic inheritance controlling a trait.
6. Sketch a graph that shows the expected variation of a named polygenic trait within a population.

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5.7 DOMINANCE INHERITANCE PATTERNS

Inheritance is not always controlled by simple Mendelian genetics, in which one allele is completely dominant over a recessive allele. There are two more kinds of dominance: **incomplete dominance** and **codominance**.

Incomplete dominance

Incomplete dominance occurs when two different alleles are present, but neither allele is completely dominant. Both alleles contribute to the phenotype, but only partially. A third intermediary phenotype is observed. If a pure-breeding red snapdragon plant is crossed with a pure-breeding white snapdragon plant, as shown in Figure 5.31, the F_1 offspring all have pink flowers. When these F_1 pink snapdragons are crossed, the F_2 offspring have flowers in the ratio of 1 red : 2 pink : 1 white. This is known as incomplete dominance or **partial dominance**, because one trait is not fully dominant over its partner, and the heterozygous phenotype (pink) is intermediate between the homozygous parental phenotypes (red and white).

A special notation is used to indicate inheritance of partially dominant traits. A suitable upper-case letter designates the gene for the trait (e.g. *C* for colour) and upper-case superscript letters indicate the alleles (e.g. C^R = red colour, C^W = white colour). Figure 5.31 is a diagram using the appropriate notation to describe inheritance of flower colour in snapdragons.

Science Photo Library/Adrian Thomas

FIGURE 5.30 In pure-breeding snapdragons, incomplete dominance of the red flower colour and white flower colour results in a pink flower colour.

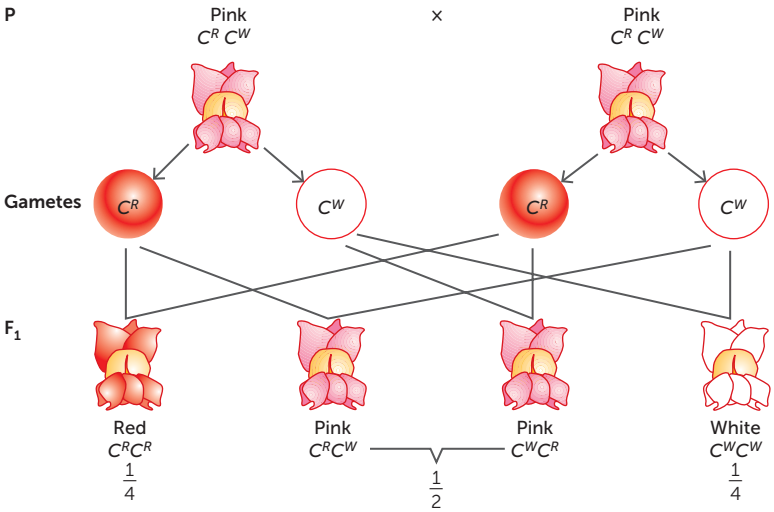

FIGURE 5.31 The result of crossing snapdragons with pink flowers among themselves is a phenotypic ratio of approximately 1 red : 2 pink : 1 white.

Codominance

Codominance occurs when two alleles are completely dominant. Both alleles are expressed and observed in the phenotype. In humans, blood group AB is a joint phenotype. The alleles that contribute to the trait are I^A and I^B. Both alleles are completely dominant and completely expressed.

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The study of certain coat colours in horses and cattle also reveals this type of dominance relationship. Both alleles in the genotype are fully expressed in the heterozygote. Such traits are said to be codominant. In shorthorn cattle, alleles for coat colour are inherited in this way, and the two alleles are expressed as red coat (C^R) and white coat (C^W). This is similar to the incomplete dominance shown in snapdragon flowers, but in this case, the offspring of the pure-breeding red and white parents have roan coats (C^WC^R), which are a mixture of red and white hair. The codominant inheritance of coat colour in shorthorn cattle is illustrated in Figure 5.32. An outline of the differences between the types of dominance relationships is provided in Table 5.4.

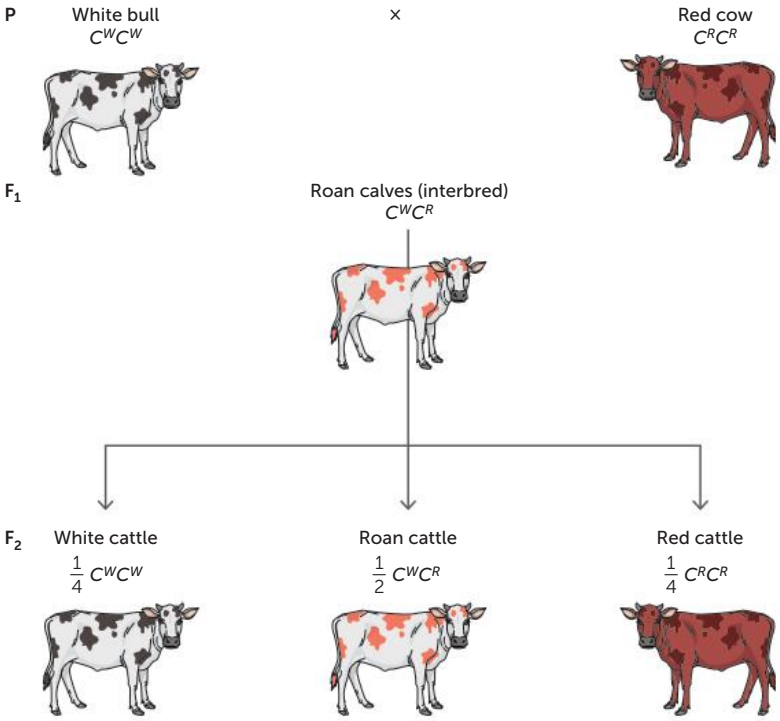
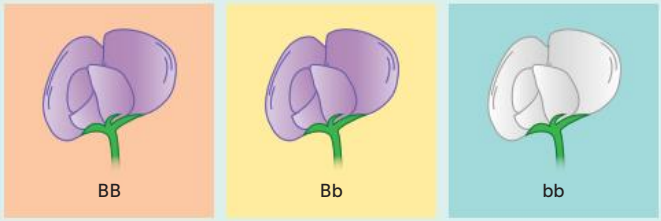


FIGURE 5.32 In shorthorn cattle, codominant inheritance causes a roan coat colour in the offspring of pure-breeding red and white parents.

Key concept

The alleles in the genotype of an individual can be classified as homozygous or heterozygous. The interaction between alleles can be expressed as phenotypes that are dominant, recessive, incompletely dominant or codominant.

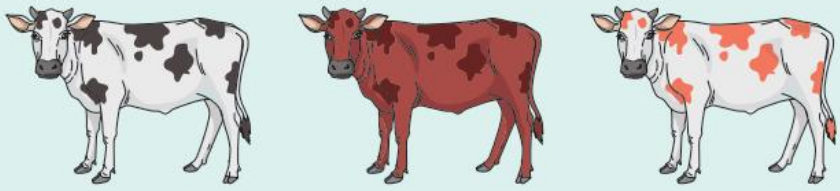
TABLE 5.4 Three types of dominance

DOMINANCE	CRITERIA	EXAMPLE
Complete dominance	One allele in a pair shows complete dominance over the other. In a heterozygote, the dominant allele is expressed and masks the recessive allele. Only the dominant trait is observed in the phenotype. The phenotypes of the heterozygote and the homozygote are indistinguishable.	 **FIGURE 5.33** Complete dominance (purple and white flower colours)

Incomplete dominance	There is an intermediate phenotype. Neither allele is completely dominant. One allele for a specific trait is not completely expressed over its paired allele.

FIGURE 5.34 Incomplete dominance (white, red and pink flower colours)

Codominance	Both alleles are completely, independently and equally expressed. Both alleles in the genotype are observed in the phenotype.



Genotype:	C^WC^W	C^RC^R	C^RC^W
Phenotype:	White	Red	Roan

FIGURE 5.35 Codominance (white, red and roan coat colours)

TABLE 5.5 Differences between the types of dominance relationships in terms of genotype and phenotype

	COMPLETE DOMINANCE	INCOMPLETE (PARTIAL) DOMINANCE	CODOMINANCE
Parents	*BB, bb*	C^BC^B, C^WC^W	C^BC^B, C^WC^W
Gametes	*Bb*	C^BC^w	C^BC^w
F_1 genotype	*Bb*	C^BC^W	C^BC^W
Phenotype	Black	Grey	Black and white patches
Gametes	*Bb, Bb*	C^BC^W, C^BC^W	C^BC^W, C^BC^W
F_2 genotype	*BB, Bb, bb*	C^BC^B, C^BC^W, C^WC^W	C^BC^B, C^BC^W, C^WC^W
Phenotype	Black White	Black Grey White	Black Black and white patches White
Heterozygote	Same as dominant	Intermediate between homozygous parents	Properties of both homozygous parents

Note: B and C^B = alleles for black coat colour; *b* and C^W = alleles for white coat colour.

Codominance and incomplete dominance
Continue investigating the inheritance of codominance and incomplete dominance using this resource.

Dominance, multiple alleles and polygenic inheritance
Inheritance factors such as dominance, multiple alleles and polygenic inheritance contribute to the phenotype of an organism. Investigate these further.

Question set 5.7

REMEMBERING

1 Contrast complete dominance, incomplete dominance and codominance.

UNDERSTANDING

2 Explain why a pink snapdragon flower phenotype is an example of incomplete dominance.

APPLYING

3 In the shorthorn cattle breed, coat colour is inherited. White and red coat colour alleles are codominant. The offspring of a cross between pure-breeding red and white parents have a roan coloured coat, due to the presence of red and white hairs.

a If a white bull is crossed with a roan cow, predict the offspring genotype and phenotype ratios and show your working out using a Punnett square.

b A farmer who breeds only roan cattle noticed one generation with 50% red and 50% roan cattle. The farmer suspected a neighbouring bull had interfered with the breeding program. Deduce the genotype and phenotype of the unknown bull.

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SCIENTIFIC LITERACY

What are Australian Sheep Breeding Values?

Scientists study the inheritance factors that affect sheep breeding. If the right sheep genes are selected, flocks will display beneficial phenotypes based on those selected genotypes. The Australian Sheep Breeding Values (ASBVs) help sheep breeders select sheep with the most desirable traits for breeding. ASBVs are based on precise genetic analysis and can be used to improve flock genetics. Only rams and ewes found to have the advantageous alleles are bred. This can result in permanent genetic changes in a population of sheep.

Desirable genetic changes in a flock result in improved phenotypes for traits such as body weight, fleece weight, growth rate, disease resistance and diameter of the wool fibre. Farmers can then sell sheep and sheep products at a higher price, leading to economic growth. This has been the case in WA.

The slower, less accurate method of **selective breeding** or **artificial selection** (the breeding of plants and animals to produce desirable traits in successive generations) uses a hypothesis-based approach. This approach involves the assumption that selected sheep that display specific phenotypes always carry the genes that code for that trait. Another assumption is that all of the offspring will have the trait. However, several genes may be required for the production of a particular trait, and not all of them will be passed to all of the offspring. Additionally, **linked genes** are inherited together more frequently because they are located near one another on the same chromosome, leading to further uncertainty. More modern techniques involve biotechnology. Using a map of the sheep genome and DNA profiles of individual sheep has led to faster and more accurate selective breeding programs. However, this technology is in its infancy.

Variants in phenotypes are affected by genetics and the environment. Scientists are trying to develop better technology in order to more accurately match the cause (genotype and environment) and effect (phenotype) for a particular trait. Currently, technology cannot tell farmers which specific alleles cause which changes to a phenotype. For polygenic traits, this will be the sum effect of all the genes affecting a particular trait. For some traits, this means studying thousands of genes for just one trait.

Source: Western Australian Agriculture Authority, Department of Primary Industries and Regional Development

Questions

1. Scientists studying the effects of specific genes on a trait try to keep other variables consistent, such as feeding levels. Can you think of other environmental factors that should be kept constant to enable results to be valid (e.g. age and sex)?
2. List sheep products that are used in Australia.
3. Describe the breeding objectives a sheep farmer might have.
4. Once breeding objectives are decided upon, farmers look for factors that can help them reach their objectives. Use your knowledge of genetics to discuss the types of crosses a farmer might employ to achieve their objectives.

Shutterstock.com/Tap10

FIGURE 5.36 Improvements in sheep genetics have led to economic growth in Western Australia.

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5.8 MODES OF INHERITANCE

There are four main modes of single-gene inheritance that are observed in human populations: autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive. The type of inheritance depends on whether the gene is found on an autosomal chromosome (autosome) or a sex chromosome. It also depends on whether the trait is dominant or recessive. Recall that traits are dominant if only one copy of the allele is required for expression of the trait. Traits are recessive if two copies of an allele are required for expression of the trait. The patterns of inheritance can be analysed using pedigrees. It is important to understand the various modes of inheritance to predict how genetic conditions are passed on from parents to offspring.

Genes and inheritance
Click through the animations step by step to understand the patterns of inheritance.

Autosomal recessive patterns

An allele on a non-sex chromosome being passed to offspring is known as **autosomal inheritance**. Alleles usually come in pairs. One gene in each pair comes from the mother, and the other gene comes from the father. Recessive inheritance of a disease means both genes in a pair must be abnormal in order to cause the disease. People with only one defective allele in the pair of alleles are called **carriers**. These people are most often not affected with the condition. However, they can pass the abnormal gene on to their children. The parents of an affected person are always at least carriers of the allele. A carrier is usually unaffected, because a dominant allele will silence the effects of the recessive allele that causes the condition.

Analysis of a pedigree can be used to determine patterns of inheritance across generations in a family. There are some universal symbols used in pedigrees (Figure 5.37).

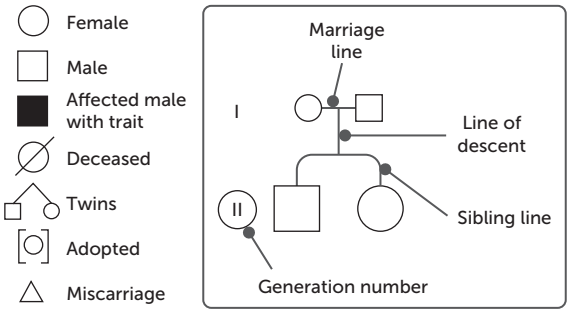

FIGURE 5.37 Pedigree symbols

Pedigree analysis 1: How to solve a genetic pedigree No. 1
Andrew Douch uses a hypothesis strategy to solve pedigree problems.

Analysis

In the pedigree illustrated in Figure 5.38, we see that two unaffected parents in the F_1 generation had an affected son. At least one of the parents would need to have had the allele causing the condition to pass it on to the son. If the allele was dominant, the F_1 parent would have been affected. As the parents are carriers, rather than being affected by the condition, the allele must be recessive.

If the mode of inheritance was **X-linked** and recessive, the P generation female parent would have the genotype X^rX^r. Her sons would have to be affected, because they would have received their one and only X chromosome from her, and she only had the allele with the condition to pass on to them. This rules out the chance of it being X-linked and confirms that the mode of inheritance is autosomal recessive.

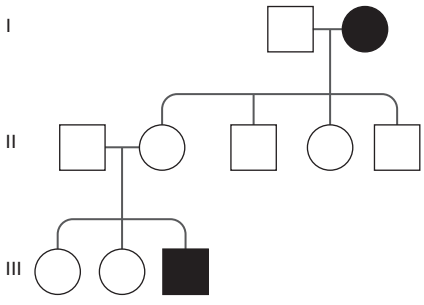

FIGURE 5.38 Pedigree for an autosomal recessive condition

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Autosomal dominant patterns

In autosomal dominant inheritance, a single dominant allele is responsible for the occurrence of a phenotype. Each affected person usually has an affected parent, and the phenotype occurs in every generation. A single copy of the affected allele is enough to cause the condition. A parent with a single copy of a dominant allele on an autosome (heterozygous) will theoretically pass it on to 50% of the offspring. If the parent is homozygous for the dominant trait, then 100% of the offspring will be affected.

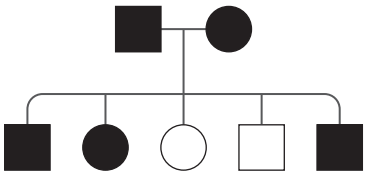


FIGURE 5.39 A pedigree demonstrating an autosomal dominant pattern of inheritance

Analyse the pedigree in Figure 5.39 to help you understand autosomal dominant inheritance patterns.

The trait affecting individuals is a dominant trait. We know it is dominant because two of the offspring are unaffected, yet both parents are affected. This means both parents were able to pass a recessive 'normal' allele to the unaffected offspring, which means both parents are heterozygous and affected. If the allele for the condition was recessive, the parents would be unaffected carriers. But can we confirm whether the mode is autosomal or X-linked? Yes! The unaffected female offspring means there must be two recessive healthy alleles, one from each parent. If the trait was carried on the X chromosome, the affected father would have passed the condition to 100% of his daughters, because he only has one X chromosome. So this pedigree tells us that the trait is autosomal.

Key concept

A pedigree is another type of visual representation that can be used to study and predict patterns of genetic inheritance.

Sex-linked inheritance

A pair of sex chromosomes is present in sexually reproducing organisms. In humans, females carry XX sex chromosomes and males carry XY sex chromosomes. Human sex is determined by the presence or absence of a Y chromosome. If a Y chromosome is present along with the X chromosome, the embryo develops into a male. If a Y is absent, the embryo develops into a female.

Genes carried on the sex chromosomes show inheritance patterns that can be described as sex-linked. Sex-linked inheritance can be detected from phenotypes that segregate differently between males and females. It is not possible to be certain about sex linkage from a pedigree chart, because autosomal traits could produce the same pattern of inheritance. At times, signs that a trait is not X-linked will become evident.

X-linked inheritance can be eliminated as a possibility if a father passes a trait to his son. Fathers cannot pass X-linked traits to their sons because their X chromosomes are passed to their daughters. They only pass their Y chromosomes to their sons. Conversely, mothers pass their X chromosomes to their sons as well as their daughters, making it possible for X-linked traits to pass from mother to sons or daughters. As males contain only a single X chromosome, the chance of X-linked inheritance of disease is higher in males than in females. This is because the dominant or recessive allele on the single X chromosome will always be expressed: there is no other allele present (see Figure 5.40). If the allele locus was on an autosome, there would be a homologous chromosome with a paired allele that could affect expression.

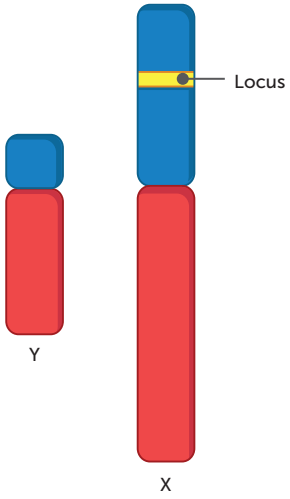


FIGURE 5.40 An allele on the X chromosome has no corresponding allele on the Y chromosome.

The locus (location) of a particular gene found on the X chromosome does not correspond to any locus on the Y chromosome; hence, it is an X-linked gene.

There are several sex-linked disorders in humans and other animals. In the same way they are used for studying autosomal traits, Punnett squares can be used to help predict genotype and phenotype ratios for sex-linked inheritance. The allele symbols are different and need to be carefully adhered to. The letters X and Y are used in the Punnett square to indicate sex-linked inheritance is being studied. Other superscripted letters are used to show whether the allele is dominant or recessive. For example, Duchenne muscular dystrophy is a condition that causes muscles to progressively weaken. Individuals with this disease often die during their teens or early 20s. Muscular dystrophy is caused by a recessively inherited mutation to a gene that codes for the protein dystrophin. The recessive allele X^d for this gene is located on the X chromosome. Possible genotypes and phenotypes for Duchenne's muscular dystrophy are listed in Table 5.6. To determine genotype and phenotype ratios, Punnett squares can be used. For example, if an unaffected female carrier married an unaffected male, can you work out the probability of their offspring being an affected male or an affected female? A Punnett square will help work out the probability.

TABLE 5.6 Possible phenotypes and genotypes for Duchenne muscular dystrophy

PHENOTYPE AND GENDER	GENOTYPE
Affected male	X^dY
Unaffected male	X^DY
Affected female	X^dX^d
Unaffected female carrier (heterozygous)	X^DX^d
Unaffected female (homozygous)	X^DX^D

Key:
Alleles
X^d = muscular dystrophy allele
X^D = normal allele
Genotype ratios: 1 X^DX^D : 1 X^DX^d : 1 X^DY : 1 X^dY

Parent cross = $X^DY \times X^DX^d$

	X^D	Y
X^D	X^DX^D	X^DY
X^d	X^DX^d	X^dY

Phenotype ratios: 1 unaffected female : 1 unaffected female carrier : 1 unaffected male : 1 affected male

There is a 50% chance of having a son. The probability of a son having the condition is 50%, so the probability of having a son with the condition is 50% × 50% = 25%.

Diseases caused by mutated genes located on the X chromosome can be inherited in either a dominant or recessive manner.

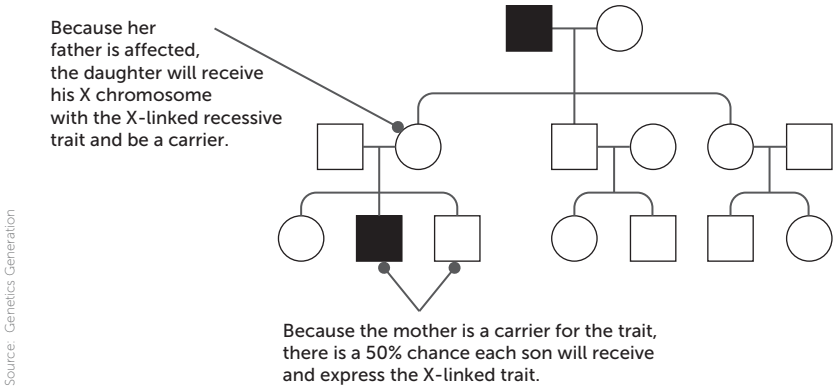
X-linked recessive patterns

The allele locus for an X-linked recessive condition is on the X chromosome. When a recessive phenotype under investigation is determined by an allele on the X chromosome, it is said to be an **X-linked recessive** phenotype. Males who have the recessive allele on their X chromosome will always express the phenotype, because they only have one X chromosome. Females will only express the phenotype when both X chromosomes have the affected allele. For a female child to be affected, the father must be affected and the mother must be either affected or a carrier. A heterozygous female will be a carrier.

Consequently, males show X-linked recessive phenotypes much more often than females do. However, this trend is not enough to confirm the mode of inheritance. Red–green colour blindness and haemophilia are two recessive conditions in humans that are transmitted to offspring through X-linked inheritance.

In this type of inheritance, a male with the phenotype cannot pass on the trait to his sons, because they inherit his Y chromosome only. His daughters will get the affected X chromosome, but they will only show the phenotype if they inherit another affected X chromosome from their mother. As with an autosomal recessive phenotype, some generations may not have any members showing the phenotype.

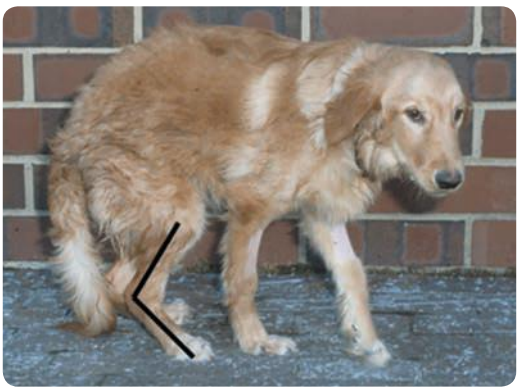
Analyse the pedigree in Figure 5.41 to help you further understand X-linked inheritance patterns.



Source: Genetics Generation

FIGURE 5.41 A pedigree demonstrating an X-linked recessive inheritance pattern

Golden retriever muscular dystrophy is also caused by an X-linked recessive allele. Dogs who suffer from this condition experience progressive degeneration of skeletal and cardiac muscles. Symptoms such as difficulty swallowing and muscle weakness (where standing is difficult) appear at around 6–8 weeks of age and worsen until the disease becomes fatal by around 6 months.



Source: Kornegay, J. N. et al. 'Canine models of Duchene Muscular Dystrophy and their use in therapeutic strategies'. Springer Science & Business Media.

FIGURE 5.42 Golden retriever with muscular dystrophy

X-linked dominant patterns

The allele locus for an X-linked dominant condition is on the X chromosome. This type of inheritance is similar to X-linked recessive inheritance, except that heterozygous females will always show the phenotype, and any individuals with the phenotype must have a parent with the phenotype. Males showing the phenotype will not pass the affected allele on to their sons (because sons must inherit their father's Y chromosome), but they will pass it on to all their daughters, who will also show the phenotype. This is because daughters always inherit their father's X chromosome. A heterozygous female is expected to pass on the allele to 50% of her offspring, regardless of their sex. The condition should appear in every generation. An affected male receives the dominant allele from an affected mother.

Y-linked patterns

If a trait is carried on the Y chromosome, it is said to be **Y-linked**. Only males are affected. Y-linked traits are rare because the Y chromosome is short and has a limited number of genes, most of which code for male sexual development. 'Maleness' in humans is determined to a large extent by the *SRY* gene carried on the Y chromosome. Other Y-linked genes are relevant to testis development and sperm production. By definition, inheritance of genes that appear on the Y chromosome is along the male line, from father to son.

Key concept

The inheritance of genes that are found on the X chromosome can be X-linked recessive or X-linked dominant, and show different patterns for males and females. X-linked recessive phenotypes are more common in males because they only have one X chromosome and will be affected regardless of whether the allele is dominant or recessive. Y-linked phenotypes are only seen in males because they are the only sex to carry a Y chromosome.

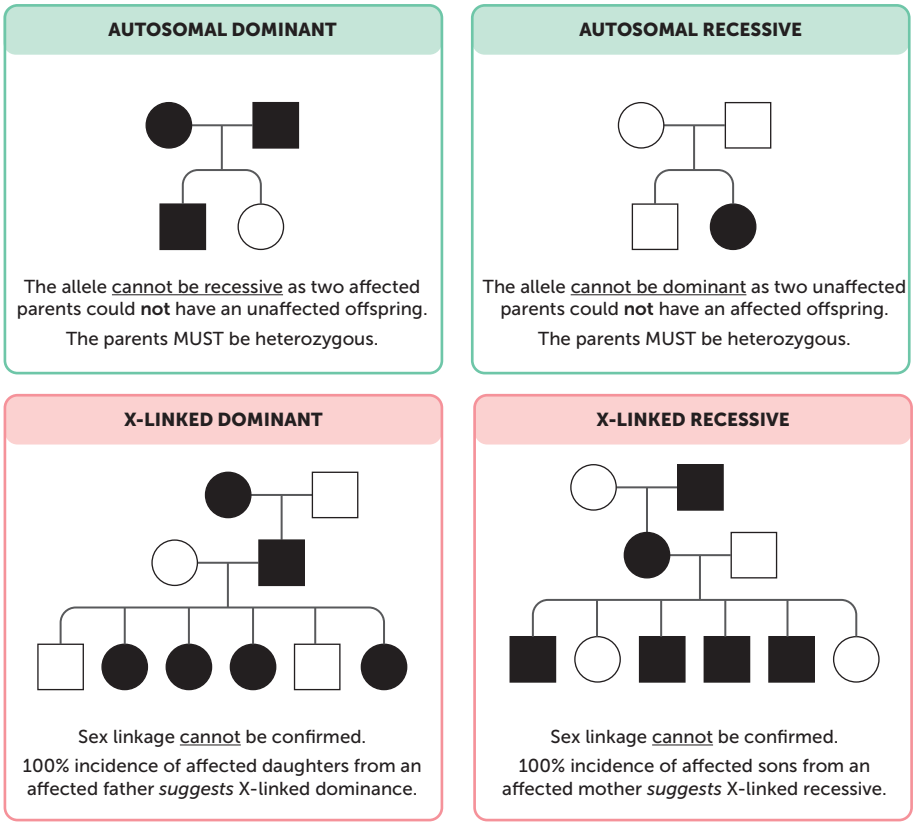


FIGURE 5.43 Not all modes of inheritance can be determined from a pedigree.

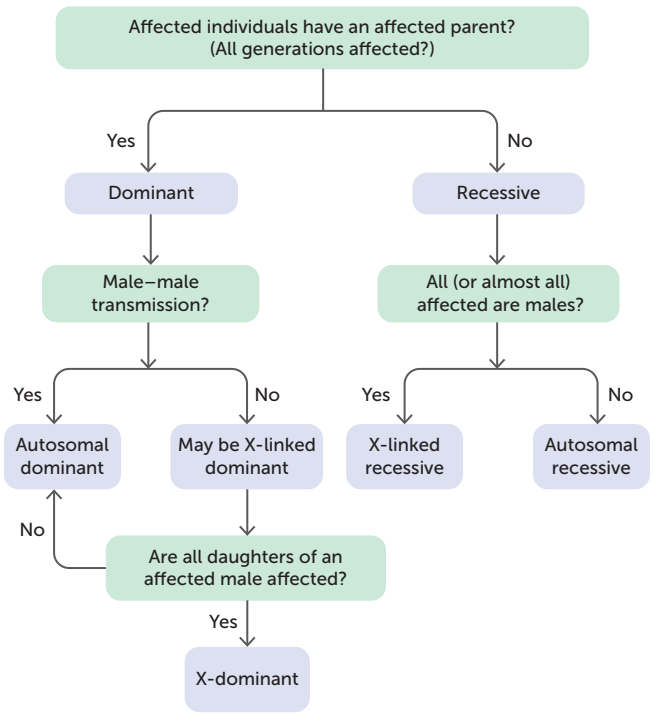


FIGURE 5.44 Using a dichotomous key to determine likely mode of inheritance from a pedigree

Remember: when you answer mode of inheritance questions, you may not always be able to confirm the mode of inheritance. You can make predictions based on the evidence and using visual representations such as Punnett squares and pedigrees; however, you cannot confirm these predictions without conducting genetic tests (e.g. using biotechnology, see Chapter 6).

Question set 5.8

REMEMBERING

1 Define:
- **a** autosomal
- **b** sex-linked inheritance
- **c** X-linked inheritance
- **d** Y-linked inheritance

2 Define carrier.

UNDERSTANDING

3 Describe and explain the occurrence of phenotypes that are:
- **a** X-linked recessive
- **b** X-linked dominant
- **c** Y-linked.

APPLYING

4 Ichthyosis is an inherited condition characterised by scaly skin. One form of the condition affects around 1 in 6000 males, but female cases are almost unknown.
- **a** What might account for the differences in occurrence of this type of ichthyosis between males and females?
- **b** From which parent would an affected male have inherited the condition?
- **c** What is the probability the affected male would pass the responsible gene on to his sons? Explain your reasoning.

5 The novel Xg blood group is an X-linked phenotype that is observed in approximately equal proportions in males and females.
- **a** Explain how an X-linked phenotype could be observed in equal proportions in both sexes.
- **b** What is the probability that Xg sons are born to a heterozygous mother with Xg and a father without Xg? What is the probability that daughters of this mother and father show the Xg trait?
- **c** What is the probability that Xg sons are born to a mother without Xg and a father with Xg? What is the probability that these parents have Xg daughters?

ANALYSING

6 After studying the pedigree in Figure 5.45, use a method like the dichotomous key or elimination method to determine the pattern of inheritance of the particular characteristic.

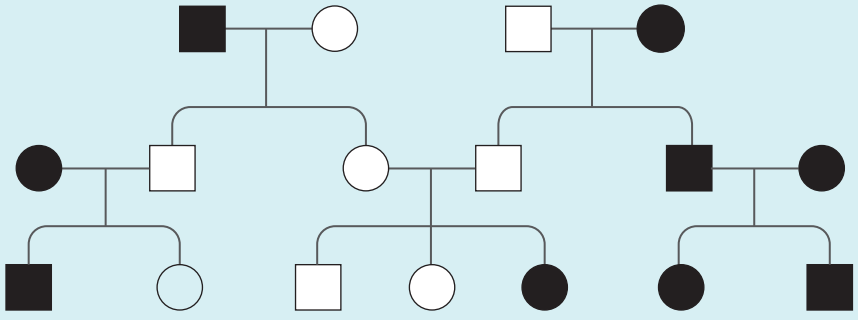


FIGURE 5.45 Pedigree for an inherited characteristic

7 The pedigree below shows the inheritance of freckles in a family. The allele for freckles (*F*) is dominant to the allele for no freckles (*f*). What is the genotype of individuals I2 and II4?

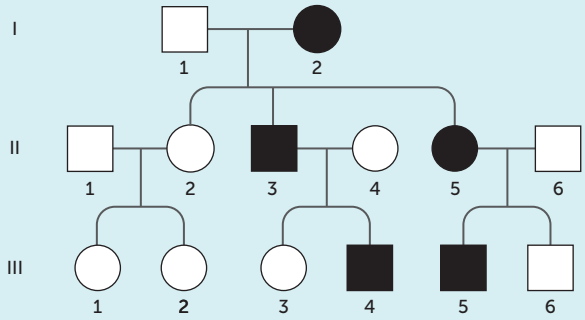


FIGURE 5.46 Pedigree demonstrating the inheritance of freckles

CHAPTER 5 ACTIVITY AND INVESTIGATION

5.1 ACTIVITY

Continuous variation: student height

Aim

To be able to distinguish continuous data from discontinuous data

You will need

Metre ruler or tape measure

What you will do

Record the heights of all students in your class or cohort, either in one communal table on the white board or in a table projected onto a screen. A minimum sample size should be around 20 students.

Studying your results

Group the data in a frequency table.

HEIGHT (CM)	FREQUENCY
150–<155	
155–<160	
160–<165	
165–<170	
170–<175	
175–<180	
180–<185	
185–<190	

Analysis of results

1. Draw a histogram of the results or use Word or Excel to create a chart.
2. Evaluate the reliability of your data in relation to how repeatable the results are and how representative your data are for the human population.
3. Your chart (graph) should look like a bell curve. Explain why that would be.
4. Calculate the mean and median.
5. Differentiate between a bar graph and a histogram.
6. You may like to collect a discontinuous data set by surveying whether the students in your class have attached or detached earlobes.
7. Tabulate the discontinuous data then construct a bar graph.
8. Explain how the results indicate that human height is caused by polygenes and not just multiple alleles of one gene.

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5.1 INVESTIGATION

Patterns of inheritance in heterozygous barley seeds

Background

Barley (*Hordeum vulgare* L.) was one of the first cultivated grains. A member of the grass family, barley is now grown in over 100 countries. Barley has 14 chromosomes and self-pollinates asexually to reproduce. A single gene with two alternative alleles controls pigmentation in barley. The dominant allele results in a phenotype with green pigmentation. The other allele produces no pigmentation, and when homozygous, it results in a white (or albino) recessive phenotype. In the heterozygote, the dominant expression of green pigment masks any expression of the allele coding for no pigment (albino).

Aim

To perform a monohybrid cross and predict phenotypic ratios

Time requirement

20 minutes

Materials

- 25 seeds of genetically selected barley
- Filter paper
- Disposable plastic Petri dish
- Plastic pipette
- Forceps
- Scissors
- PPE: lab coats, safety glasses, gloves

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Some people may be allergic to particular seeds.	Do not eat seeds. Be aware of any known allergies.

Procedure

1. Place a piece of filter paper into the bottom half of a Petri dish. Trim the paper as necessary so it lies flat.
2. Soak the filter paper with tap water using a pipette. Remove or drain any excess water that is not absorbed by the paper.
3. Sprinkle the seeds evenly over the moistened paper in the Petri dish. Using your forceps, ensure the seeds are evenly spread out (approximately 1 cm apart).
4. Place the Petri dish with seeds on a bench with sufficient access to sunlight, keeping them at room temperature.
5. Rehydrate the seeds twice a day using a pipette to prevent them from drying out. This process of twice daily rehydration should continue until the barley seedlings reach 2 cm in height, which will take approximately 1 week.
6. Propose a hypothesis about the phenotypic ratio you would expect to see using a Punnett square (monohybrid cross), where 'A' represents pigment produced (green, dominant) and 'a' represents no pigment produced (white, recessive albino).

	A	*a*
A		
a		

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7 Observe the seedlings at the end of 1 week. Some seedlings will be pale (albino) with little or no green pigment. Other seedlings will have green areas forming. When nearly all the seedlings have germinated, count the number of green seedlings and the number of albino seedlings. Record your results and then contribute your individual data to the class data.

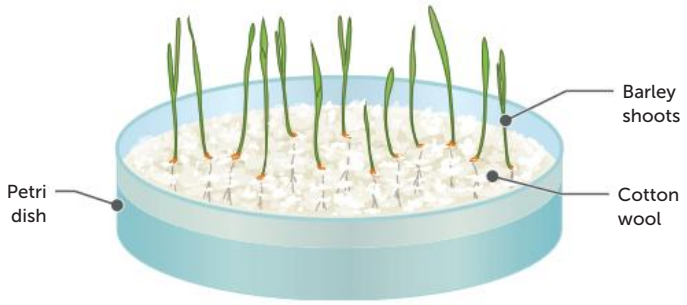

FIGURE 5.47 Set-up to test for patterns of inheritance in heterozygous barley seeds

Results

1 Record your individual and class data in the table below:

	TOTAL NUMBER OF SEEDLINGS	NUMBER OF SEEDLINGS OF EACH COLOUR	
		GREEN	ALBINO
Individual data			
Class data			

2 Calculate the ratio of green seedlings to albino seedlings for:
   a your individual data
   b the class data.
3 How do your individual data compare with the ratio for the class data?

Discussion

1 What is one limitation of this procedure?
2 How could you improve the reliability and validity of the data produced in this investigation?
3 Was your predicted ratio (based on your Punnett square) correct?
4 Were there any inconsistencies in the results? If so, explain why they may have occurred.
5 Based on the class data, what would you assume is the mode of inheritance for green pigmentation in barley? Would your answer be the same if it was based only on your individual data?
6 Homozygous green barley plants are indistinguishable visually from heterozygotes. To identify the genotype of an individual plant showing the dominant characteristic, a geneticist undertakes a test cross. Describe a test cross.
7 If presented with 120 seedlings, approximately how many would you expect to be green? Show your working out.

Conclusion

Summarise your findings in this investigation, commenting on your hypothesis about the mode of inheritance for pigmentation in barley.

Taking it further

1 In this investigation, you have conducted a monohybrid cross. What type of investigations could you conduct to demonstrate
   a a dihybrid cross
   b a sex-linked cross?
2 Calculate the chi-square value for this experiment. Do your observed frequencies deviate significantly from the expected frequencies for this cross?

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WS
Chapter 5 Activity sheet

CHAPTER 5 SUMMARY

- The genotype of an organism is the combination of alleles it has for a particular gene or multiple genes.
- For any gene, an individual may have two alleles that are identical (homozygous) or different (heterozygous).
- For single-gene inheritance, the presence or absence of certain alleles determines an organism's phenotype. Phenotypes may be described as dominant, recessive, codominant or incomplete.
- In a monohybrid cross between two pure-breeding (homozygous) parents:
  - the F_1 offspring are all heterozygous and show the dominant phenotype
  - in a subsequent cross between F_1 individuals, the F_2 offspring are predicted to show dominant and recessive phenotypes in the ratio of 3:1.
- In a dihybrid cross between two parents pure-breeding for two independently assorting characteristics:
  - the F_1 offspring are heterozygous for both traits and show both dominant phenotypes
  - in a subsequent cross between F_1 individuals, the F_2 offspring are predicted to show four combinations of phenotypes, dominant–dominant : dominant–recessive : recessive–dominant : recessive–recessive in the ratio of 9:3:3:1.
- Punnett squares are a convenient way to represent crosses, and to predict the resulting genotypes and phenotypes and their proportions.
- Observed phenotypic ratios for any cross may vary from those predicted by a Punnett square. This is due to:
  - random assortment of alleles on chromosomes during meiosis
  - the fertilisation of random gametes.
- A test cross can be used to determine the genotype of an individual displaying the dominant phenotype. It involves crossing the individual with a homozygous recessive mate and analysing the offspring.
- Discontinuous variation is displayed by phenotypes that are governed by a single gene. Continuous variation is shown for phenotypes that are governed by polygenic inheritance.
- Sex-linked inheritance is suggested when there is an unequal phenotypic ratio between males and females, although this does not confirm it.
- X-linked recessive phenotypes are more common in males than in females. An affected daughter must have an affected father.
- X-linked dominant phenotypes are observed in all affected females and males.
- A pedigree is a helpful representation of inheritance; however, it cannot confirm the mode of inheritance.
- Y-linked phenotypes are exclusively male because the allele is only present on a Y chromosome.

CHAPTER 5 GLOSSARY

Allele One of various versions of the same gene (at the same locus) distinguished by small differences in its DNA sequence

Artificial selection The breeding of plants and animals to produce desirable traits in successive generations; also known as selective breeding

Autosomal inheritance The passing on of a trait through a gene located on an autosome, a chromosome that is not a sex chromosome

Autosomal trait A trait coded for by a gene on an autosome, a chromosome that is not a sex chromosome; a gene of this kind is called autosomal

Carrier In reference to a genetic disease, a carrier is a healthy, heterozygous organism carrying an allele for a recessive phenotype; the organism may transmit the recessive allele and its associated phenotype to its offspring

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Codominance A state in which both alleles of a heterozygous individual are fully expressed in the phenotype

Continuous variation Variation in a phenotype characteristic that shows a smooth range; this occurs when a trait is controlled by many genes; when graphed, such variation forms a bell-shaped (normal) curve

Critical thinking The analysis and evaluation of a concept using logic and connection between ideas

Dihybrid cross A cross between two organisms that are heterozygous at two gene loci

Discontinuous variation Variation in a characteristic that shows two or just a few clearly distinct phenotypes

Dominant A phenotype that requires only one copy of its allele for it to be expressed in an individual

First filial generation (F_1) The first generation of offspring produced from a cross between two parents (P)

Gene A set of instructions that specifies the structure of a protein

Genetics The study of the mechanisms and patterns of inheritance associated with the transmission of coded chemical instructions from one generation to the next

Genotype The specific combination of alleles for a particular gene locus belonging to an individual or cell

Heredity The study of inheritance; the genetic transmission of characteristics from one generation to the next

Heterozygous A genotype with two different alleles for a single gene locus

Homozygous A genotype with two identical alleles for a single gene locus

Incomplete dominance The state in which a heterozygous individual has a phenotype that is intermediate between those of the corresponding homozygous individuals

Independent assortment Random orientation of maternal and paternal homologous chromosomes at the equator during metaphase I, resulting in random combinations of alleles in the gametes at the conclusion of meiosis

Inheritance The genetic acquisition of characteristics by offspring from their parents

Linked genes Genes or alleles that are inherited together more frequently because they are located near one another on the same chromosome

Monohybrid An organism that is heterozygous with respect to a single gene

Monohybrid cross A cross between two monohybrids (see **monohybrid**); only one gene is involved, and the cross is between two organisms that are heterozygous at one gene locus for a dominant and a recessive allele

Multiple alleles The term given when three or more alleles of a gene exist among members of a population

Non-homologous chromosomes Chromosomes that do not belong to the same pair; they contain different sets of genes

Parental generation (P) Two individuals or lines that represent the start of a breeding experiment; their offspring are the F_1 generation

Partial dominance See **incomplete dominance**

Phenotype The actual form taken by a specific feature in a particular individual, based on their genotype and influenced by the environment; it can be used in reference to particular traits or characteristics, or to the overall form of an individual

Polygene A gene whose alleles have a small, additive effect on a phenotype; many polygenes together contribute to continuous variation in a phenotype

Polygenic inheritance Transmission between generations of characteristics that are controlled by polygenes (see **polygenes**)

Punnett square A Punnett square is a table that displays all the possible offspring genotypes given the parent alleles that can pair up at fertilisation; it can be used to determine the likelihood of producing offspring with particular genotypes and phenotypes from known parental genotypes, and sometimes to deduce parental genotypes given offspring genotype and phenotype percentages

Pure-breeding A line of organisms that always produce offspring with the same phenotype when crossed with one another

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Recessive A phenotype that requires two copies of its allele in an individual in order to be expressed

Second filial generation (F_2) Offspring of the F_1 generation; the second generation produced from a cross between two homozygous parents (P)

Selective breeding A process by which humans domesticate animals or plants by purposely choosing individuals with the most desirable characteristics as parents for each successive generation of breeding

Sex-linked Describes a gene located on a sex chromosome

Sex-linked trait A trait inherited on a sex chromosome (X or Y chromosome); the gene of interest on a sex chromosome is described as sex-linked

Test cross A technique used by geneticists in which an individual whose genotype is unknown for a dominant phenotype (it could be homozygous dominant or heterozygous dominant) is crossed with an individual that is homozygous recessive at the locus in question

Trait An inheritable characteristic; phenotype

X-linked Related to a gene located on the X chromosome

X-linked recessive When a phenotype is determined by a recessive allele on the X chromosome

Y-linked Related to a gene located on the Y chromosome

CHAPTER 5 REVIEW QUESTIONS

Remembering

1 Describe the relationship between the following terms:
   - **a** gene and allele
   - **b** genotype and allele
   - **c** genotype and phenotype.

2 Match each item in the first column with a description in the second column. Each item can only be used once.

Heterozygous	Only one copy of the allele is required for the phenotype to be observed.
Homozygous	Two different alleles are both fully expressed in the phenotype.
Recessive	The phenotype is intermediate between each of those determined by two different alleles.
Dominant	Two copies of the same allele are present for a particular gene locus.
Codominant	Two different alleles are present for a particular gene locus.
Partially dominant	Two copies of the allele are required for the phenotype to be observed.

Understanding

3 Explain what 'pure-breeding' means. Why was it important for Mendel to use pure-breeding plants in his experiments?

4 Explain why siblings are not identical, even though they inherit their chromosomes from the same parents.

5 Explain why none of the offspring of a tall pea plant and a short pea plant are of intermediate height.

6 Distinguish between the effects of random assortment of alleles and linked alleles on phenotype, and explain what accounts for these differences.

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Applying

7 Two grey rats are mated. Half the offspring are grey, one-quarter are white and one-quarter are black.
   a Assign the alleles for coat colour.
   b Describe the genotypes of the parents and the offspring.
   c What kind of dominance is this?

8 There are four possible phenotypes for ABO blood groups in humans: A, B, AB and O. The most common of these, O, is the recessive phenotype. The phenotypes are determined by three alleles, of which I^A and I^B are codominant and i is recessive to both.
   a Write each possible genotype and the corresponding phenotype.
   b If a woman is heterozygous with blood type A and a man is heterozygous with blood type B, predict their children's possible blood type(s) and the probability of each. Support your conclusions with a Punnett square.

9 Some fruit flies carry an X-linked recessive gene for white eye colour, over which the red-eyed phenotype is dominant. Predict the proportion of red-eyed and white-eyed offspring and their gender resulting from a cross between an F_1 female and an F_1 male. Use a Punnett square to support your prediction.

10 In mice, coat colour is determined by an autosomal gene, and pink coat colour is dominant to brown. Dwarfism is caused by an X-linked recessive allele. If a brown female dwarf mouse mates with a pure-breeding pink male of normal size, what will the phenotypic ratios in each gender be in the F_1 and F_2 generations?

11 In cherry tomatoes, a tall vine is dominant to a dwarf vine, round fruit is dominant to pear-shaped fruit, and red fruit is dominant to yellow fruit. If you crossed a pure-breeding tall, round-fruited, red-fruited plant with a short, pear-shaped-fruited, yellow-fruited plant, what would you expect to be the appearance of the F_1 generation? Assuming that the genes controlling these three characteristics are inherited independently, what are the possible combinations of genes in the gametes of the F_1 generation?

Analysing

12 The snapdragon (*Antirrhinum majus*) can show a condition called 'aurea', in which the leaves appear a golden colour instead of green. A pair of aurea snapdragons with golden leaves was crossed and they produced 101 offspring: 67 with golden leaves and 34 with green leaves. Draw a Punnett square for the cross and use the data to explain how the ratio seen in the F_2 offspring could have arisen.

13 A test cross of fruit flies with curly wings and red eyes produces offspring with red eyes, half of which have curly wings, and half, straight wings. Identify the genotype of the original red-eyed fruit fly with curly wings and provide evidence to support your conclusion.

14 A male pure-breeding fruit fly with a standard brown body is crossed with a female pure-breeding fruit fly with a yellow body. All of the male offspring have yellow bodies, whereas all of the female offspring have brown bodies.
   a Explain where the gene is located.
   b Predict the proportions of the phenotypes in the offspring produced by crossing the F_1 fruit flies.

Evaluating

15 Discuss the benefits and limitations of studying Mendelian inheritance patterns in humans.

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Creating

16 Would you consider most phenotypes to be caused by a single gene or polygenic? Discuss the observations you would cite in support of your point of view.

PRACTICE EXAM QUESTIONS

1 Like mammals, the fruit fly *Drosophila* has an XY system of sex determination. *Drosophila* usually have large, round eyes, but a dominant allele at the Bar gene on the X chromosome causes small, narrow eyes, called 'Bar eyes'. If a male with Bar eyes is crossed with a female with normal eyes, then:

- **A** half of the female offspring will have Bar eyes but none of the males
- **B** half of the male offspring will have Bar eyes but none of the females
- **C** all of the male and female offspring will have Bar eyes
- **D** all of the female offspring will have Bar eyes but none of the males.

[Q10 2019 SCSCA]

2 Tail length in mice is a polygenic trait. This means that variation in tail length in a population of mice will be:

- **A** discontinuously distributed and controlled by the alleles at multiple genes
- **B** discontinuously distributed and controlled by the alleles at a single gene
- **C** continuously distributed and controlled by the alleles at multiple genes
- **D** continuously distributed and controlled by the alleles at a single gene.

[Q17 2018 SCSA]

3 In watermelons, fruit bitterness is determined by two alleles at a gene, and the allele for bitter fruit is dominant over the allele for sweet fruit. A plant that is heterozygous for these two alleles is crossed with a plant with sweet fruit. The expected fruit phenotypes in the progeny of these plants is:

- **A** all bitter fruit
- **B** all sweet fruit
- **C** 50% bitter fruit and 50% sweet fruit
- **D** 75% bitter fruit and 25% sweet fruit.

[Q11 2017 SCSA]

4 Like humans, cattle have an XY system of sex determination. In cattle, a disease called AED is caused by a recessive allele at a gene on the X chromosome. Two cattle that do not have AED disease produce a male offspring with AED disease and a female without AED disease. What is the probability that the female offspring is a carrier of AED, i.e. has one copy of the AED allele?

- **A** 100%
- **B** 50%
- **C** 25%
- **D** 0%

[Q28 2017 SCSA]

5 In domestic cats, a dominant allele at an autosomal gene results in extra toes, while a recessive allele results in a normal number of toes. Two cats with extra toes, both heterozygous for the allele that results in extra toes, are crossed and produce a litter of kittens. Cats have an XY system of sex determination like humans. What is the probability that the first-born kitten will be a male with a normal number of toes?

- **A** 0.750
- **B** 0.375
- **C** 0.250
- **D** 0.125

[Q30 2016 SCSA]

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6 Albinism is an inherited trait that results in a lack of colour in the eyes and fur of mammals, including guinea pigs. Non-albino guinea pigs have coloured fur.

- **a** A non-albino male and a non-albino female guinea pig were crossed and produced a litter containing some albino and some non-albino offspring.
- **b** Both male and female albino offspring were produced in the cross described in part a. On this basis, explain in words why albinism cannot be a sex-linked trait in guinea pigs. (4 marks)
- **c** **i** What is the probability of obtaining an albino offspring from the cross described in part a? (1 mark)
  - **ii** Draw a Punnett square to show how you obtained your answer in part c i. Indicate clearly the genotypes and phenotypes of the offspring. (4 marks)

[Q33a,b 2018 SCSA]

Use the following information to answer questions 7–9.

In the maize plant, the texture of the seed is either smooth or wrinkled. Seed texture is determined by the alleles at a single gene. A plant with wrinkled seeds was crossed with a plant with smooth seeds (the Parental generation). The parent plant with smooth seeds was a homozygote for the seed texture gene. All of the offspring of the cross had smooth seeds (the F_1 generation). Individuals in the F_1 generation were crossed with one another to produce a second generation (the F_2 generation).

7 On the basis of the above information, what seed phenotypes would be present in the F_2 generation and in what proportions would they occur? Show your workings. Use S1 to indicate the allele that produces smooth seed and S2 to indicate the allele that produces wrinkled seed. (5 marks)

[Q31c 2016 SCSA]

8 The vinegar fly, *Drosophila melanogaster*, has an XY system of sex determination like humans. White eyes, due to the eyes lacking pigment, is determined by a gene on the X chromosome. The allele that causes white eyes is recessive to the allele for normal (pigmented) eyes. List all possible genotypes for the white eyes gene for the following flies. Use '*w*' to designate the white eyes allele and '+' to indicate the allele that produces normal eyes. (4 marks)

- **1** a male with white eyes
- **2** a male with normal eyes
- **3** a female with white eyes
- **4** a female with normal eyes

[Q31d 2016 SCSA]

9 Explain what a polygenic trait is. Give a specific example. (3 marks)

[Q31e 2016 SCSA]

10 Distinguish between a dominant allele and a recessive allele (2 marks)

[Q34c(ii) 2015 SCSA]

6 BIOTECHNOLOGY – ITS TOOLS AND TECHNIQUES

CHAPTER 6 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Do you know what a genome map is and how it can be used in animal and plant conservation?
2 Can you describe some of the biotechnology tools used to construct a personalised DNA profile?
3 Are our agricultural biotechnology techniques effective enough to feed our world's population? How can they be improved?

SCIENCE UNDERSTANDING

» DNA sequencing enables mapping of species genomes; DNA profiling identifies the unique genetic makeup of individuals

SCIENCE AS A HUMAN ENDEAVOUR

» Transgenic organisms have been engineered for desirable traits, including resistance, faster growth rate, greater product quality and yield, and tolerance to adverse environmental conditions

SCIENCE INQUIRY SKILLS

» Conduct investigations, including the use of probabilities to predict inheritance patterns, real or virtual gel electrophoresis, and population simulations to predict population changes, safely, competently and methodically for the collection of valid and reliable data

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9780170452922

6.1 INTRODUCTION TO BIOTECHNOLOGY

The term **biotechnology** refers to the tools and techniques used on organisms or the products of organisms to make a product or solve a problem for human benefit. Biotechnology combines knowledge of biology and technology and can be used to improve our lives and the health of our planet. For over 10 000 years, humans have used traditional forms of biotechnology to selectively breed plants and animals.

More recent advancements in biotechnology have enabled scientists to change the genetic sequence in living things, enabling them to create new plants and domestic animals with enhanced phenotypes. Modern DNA biotechnology uses tools (e.g. **restriction enzymes**, **plasmids**, **vectors** and microarrays) and techniques (e.g. **DNA sequencing**, **polymerase chain reaction (PCR)**, **gel electrophoresis** and **recombinant DNA technology**) to extract, analyse and manipulate DNA to make useful products. Biotechnology is used in many industries, such as environmental conservation, the agriculture sector, food technology, forensics, and medical devices and diagnostics. It also plays an important role in producing clean technologies, including recycling and renewable energy. Along with technological developments comes the added responsibility to consider economic, social and ethical issues.

Traditional biotechnology

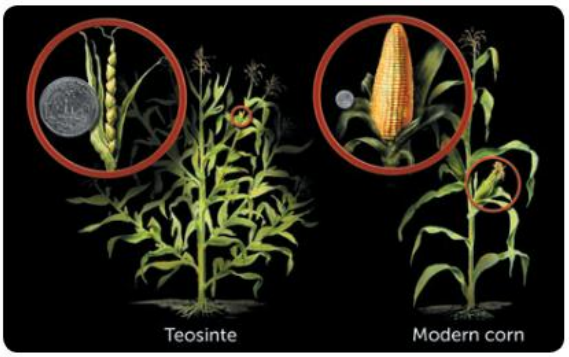
Traditional biotechnology involves purposefully selecting organisms with desirable and useful traits for breeding and cultivation. The domesticated dairy cows you see in farmers' fields today bear little resemblance to the ancient aurochs from which they are descended. And the large, yellow corn you eat is the domesticated version of a small wild grass called teosinte. In both cases, the organisms identified as having more desirable phenotypes were selected for producing offspring with those favourable phenotypes. Modern biotechnology methods also involve purposefully selecting organisms with desirable and useful traits, but results can sometimes be achieved very quickly. Selective breeding makes very small changes to phenotypes over many years. The differences we see in the cows and corn of today have taken thousands of years to produce.

Auroch (wild cow: ancestor of all domesticated cattle)

Domesticated dairy cow

iStock.com/Nerthuz

FIGURE 6.1 The selectively bred domestic dairy cow had a wild ancestor.



Science Photo Library/NICOLLE RAGER FULLER/ NATIONAL SCIENCE FOUNDATION

FIGURE 6.2 Selectively bred corn: ancient teosinte and modern corn

Key concept

Both traditional and modern biotechnology methods can be used to improve our lives. Modern biotechnology builds upon our accumulated knowledge of the structure and functioning of DNA to develop new varieties of organisms much more quickly than traditional methods.

6.2 DNA TOOLS USED IN BIOTECHNOLOGY

Modern biotechnology involves processing and manipulating DNA. Just as in the construction of buildings, tools and techniques are used for specific purposes. Biotechnology has its own set of specialised tools and techniques, which are mostly derived from organisms. These include tools for synthesising, cutting and pasting DNA, along with tools and techniques for viewing and analysing DNA. Many of these tools are enzymes.

TABLE 6.1 DNA tools

Restriction enzymes	Enzymes that cut DNA molecules at **recognition sites** (specific nucleotide sequences), usually 4–8 bases long. Naturally occurring restriction enzymes protect bacteria by cutting foreign DNA and then removing invading organisms. There are two types of cuts: **1** **sticky ends** (one strand has overhanging complementary bases, specific) **2** **blunt ends** (no overhang, non-specific).
DNA ligase	An enzyme that uses complementary base pairing to seal and reassemble DNA strands in the process of ligation. DNA ligase catalyses a strong covalent bond, closing up the sugar–phosphate backbone to hold the two strands of DNA together. Useful for recombinant DNA technology.
DNA polymerase	A class of enzymes that synthesises new strands of DNA based on a template strand and according to complementary base-pair rules. DNA polymerase adds free nucleotides one at a time. As it moves along a template strand, it attaches the complementary nucleotide to make a new strand. This is used in amplifying DNA during PCR.
Primers	Short fragments of single-stranded DNA or RNA. Primers assist in the synthesis of new strands of DNA by acting as a signal for the polymerase to begin synthesis.

FIGURE 6.3 Restriction enzymes act like scissors.

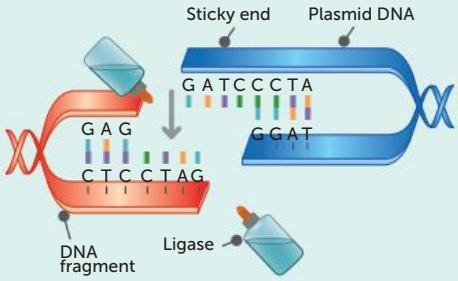


FIGURE 6.4 DNA ligase acts like glue.

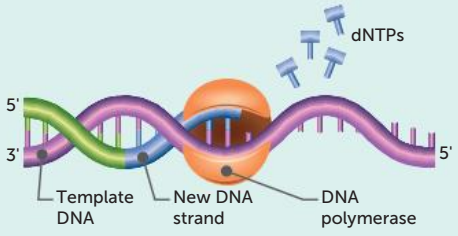


FIGURE 6.5 Polymerase enzymes can attach new nucleotides.

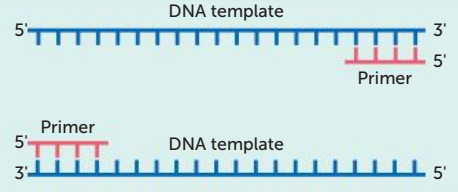


FIGURE 6.6 Primers are short fragments made of nucleotides.

Restriction enzymes: cutting DNA

One of the essential requirements in **genetic engineering** is the ability to cut segments of DNA at known sequences. The cutting tools used are enzymes known as **restriction endonucleases** ('endo' = within, 'nuclease' = an enzyme that cleaves nucleic acids), or restriction enzymes. These are like molecular scissors, cutting DNA molecules into smaller pieces, called **restriction fragments**, in a controlled way. DNA cut with restriction enzymes is often said to be 'digested' by the enzymes. Restriction enzymes only cut specific sequences of DNA, known as **restriction sites**. Different restriction enzymes have different restriction sites, though some restriction enzymes do share restriction sites with other restriction enzymes. Restriction enzymes are not specific to a species, but to a specific sequence of bases.

Restriction enzymes occur naturally in bacteria, where they cleave (cut) foreign DNA from invading viruses, thus destroying any potential threat. Restriction enzymes are named according to the bacterial strain from which they are derived. The first restriction enzyme was isolated from *Escherichia coli* RY13 strain and was thus named EcoRI. Table 6.2 lists a number of common restriction enzymes and their sources, but there is no need to memorise the table's contents.

TABLE 6.2 Common restriction enzymes and their restriction sites

ENZYME	BACTERIAL SOURCE	RESTRICTION SITE	AFTER CUTTING
EcoRI	*Escherichia coli*	↓ 5' GAATTC3' 3' CTTAAG5' ↑	5'G AATTC3' 3'CTTAA G5'
HindIII	*Haemophilus parainfluenzae*	↓ 5' AAGCTT3' 3' TTCGAA5' ↑	5'A AGCTT3' 3'TTCGA A5'
AluI	*Arthrobacter luteus*	↓ 5' AGCT3' 3' TCGA5' ↑	5'AG CT3' 3'TC GA5'
BamHI	*Bacillus amyloliquefaciens* H	↓ 5' GGATCC3' 3' CCTAGG5' ↑	5'G GATCC3' 3'CCTAG G5'

To date, almost 4000 different restriction enzymes have been identified. Although each enzyme recognises a specific sequence of between four and eight nucleotide base pairs (bp) of double-stranded DNA, multiple enzymes isolated from different organisms can recognise the same sequence. Restriction enzymes bind to their restriction site and cut the double-stranded DNA at that point. The cuts may form either overhanging steps, called sticky ends, which leave some nucleotides exposed (Figure 6.7), or blunt ends (Figure 6.8), in which the cut has occurred at the same position in each strand of the DNA and there are no overhanging strands.

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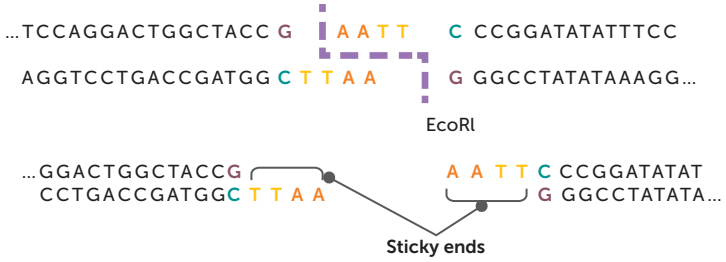

FIGURE 6.7 Sticky ends produced by cutting DNA with the restriction enzyme EcoRI

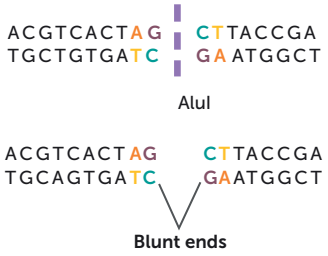

FIGURE 6.8 Blunt ends produced by cutting DNA with the restriction enzyme AluI

TABLE 6.3 Differences between sticky end restriction enzymes and blunt end restriction enzymes

FEATURE	RESTRICTION ENZYMES THAT PRODUCE BLUNT ENDS	RESTRICTION ENZYMES THAT PRODUCE STICKY ENDS
Exposed nucleotides	Blunt ends do not have exposed nucleotide bases at each end. The ends of the remaining DNA and of the removed fragment are blunt.	Sticky ends contain an overhanging, single-stranded sequence of exposed nucleotides (known as a recognition site) that is ready for complementary base pairing.
Specificity	Non-specific	Specific
Advantage	Fragments can join with any other blunt end fragment.	Fragments can join efficiently with a desired fragment that is cut with the same restriction enzyme. They can produce specific products at a faster rate.

DNA ligase: recombining DNA

At times, molecular biologists may want to combine two fragments of DNA. DNA ligase is an enzyme that is used to join pieces of DNA together. DNA ligase acts by forming a phosphodiester bond between two fragments of DNA. It joins the 3' hydroxyl end of one nucleotide to the 5' phosphate end of another nucleotide.

Two DNA fragments that have been cut with the same enzyme will have identical sticky ends, and thus complementary bases will be exposed. DNA ligase can then be used to recombine these two fragments, even if they are from two unrelated organisms. For example, EcoRI can be used to cut both human DNA and bacterial plasmid DNA, leaving sticky ends that are complementary, and increasing the chance of two complementary ends coming together (Figure 6.9). Fragments with blunt ends can also be joined by DNA ligase, but without the complementary overhangs there is no formation of hydrogen bonds to stabilise the structure, and the process is much less efficient. The technology that recombines DNA from different sources to modify the DNA sequence is called recombinant DNA technology.

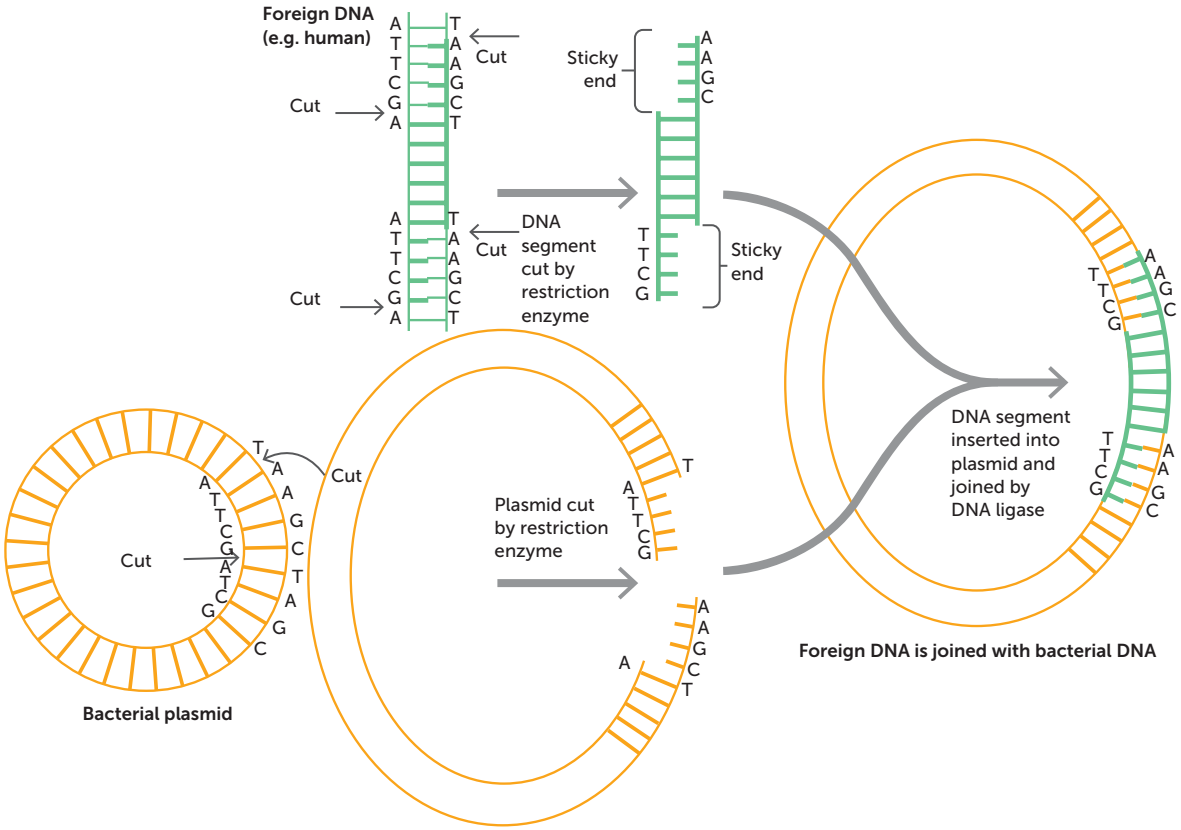

FIGURE 6.9 DNA ligases are used to join two pieces of DNA (one from a foreign source). Joining works most effectively when the two pieces of DNA have complementary sticky ends.

Primers: marking DNA

Primers are short, single-stranded (chemically synthesised) DNA molecules used in the DNA technique called PCR. During this process, DNA is synthesised using special DNA polymerase enzymes in a step called extension. The polymerase enzymes do not know where to start extending by themselves. Therefore, primers are used to mark the two ends of a target sequence of DNA that the experimenter wishes to amplify (make multiple copies of) in a test tube. The primer's sequence of nucleotides is complementary to specific sequences of DNA at either end of the target sequence. Primers join at the 3' end of the template strand, enabling extension/synthesis to be in a 5' to 3' direction. Note that the primer for initiating synthesis in DNA replication is made out of RNA.

A primer is synthesised by an enzyme called primase. This can be done in a laboratory, and scientists can manufacture primers that best suit their needs. The synthesis of a primer is necessary because the enzymes that synthesise DNA, which are called DNA polymerases, can only attach new DNA nucleotides to an existing strand of nucleotides. The primer therefore serves to prime and lay a foundation for DNA synthesis.

Key concept

Biotechnology tools include the use of restriction enzymes (often from bacteria) to cut DNA, ligases to recombine DNA (using base-pairing rules), and primers to mark the strand of target DNA that is needed.

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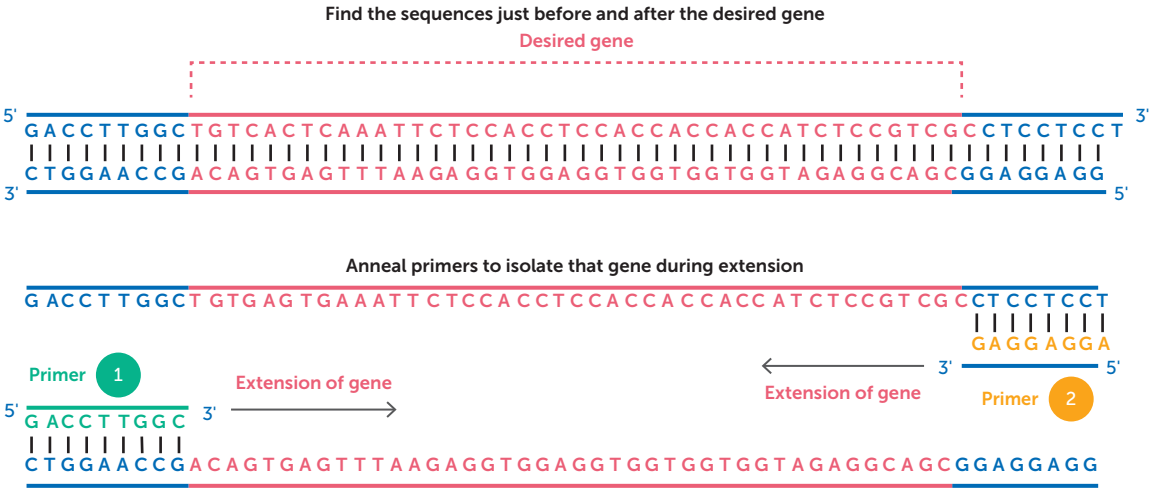


FIGURE 6.10 Primers anneal (attach by base pairing) to the beginning of a target gene.

Question set 6.2

REMEMBERING

1 1 Recall the purpose of the following tools:
   - **a** restriction enzymes
   - **b** DNA ligase
   - **c** primers.
2 Define recognition site.
3 Recall the bond that forms when DNA ligase joins two fragments of DNA.

UNDERSTANDING

4 Contrast sticky-end and blunt-end cuts made by restriction enzymes.
5 Explain why primers are needed when DNA is being synthesised.

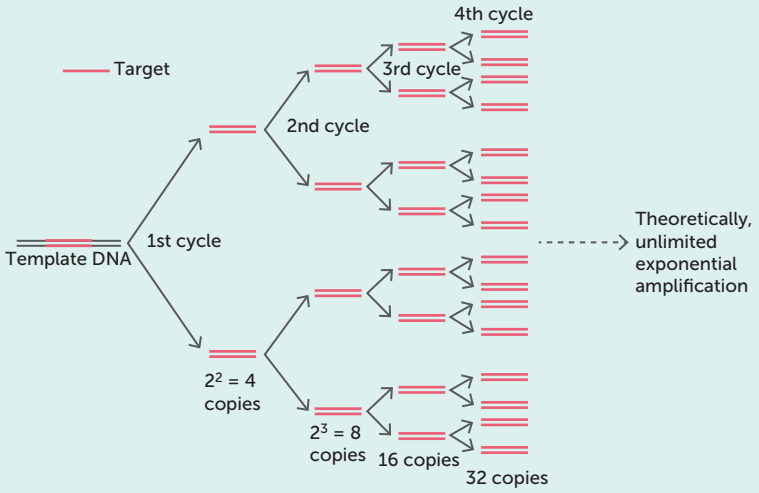
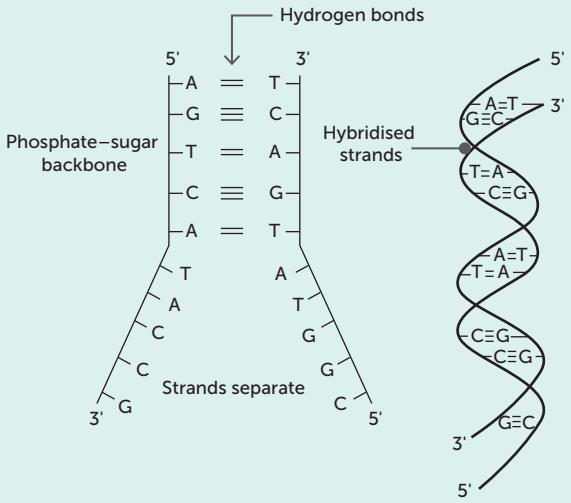
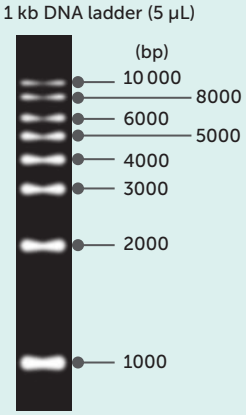
ANALYSING

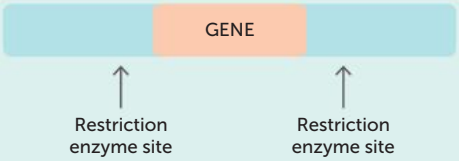
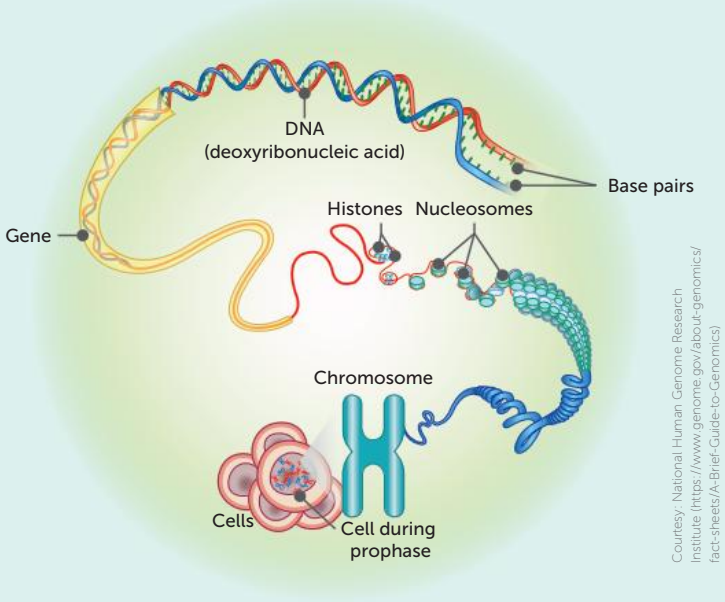
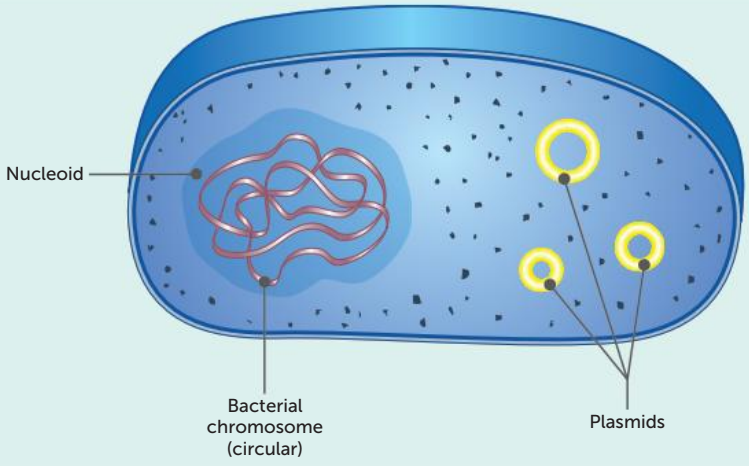
6 If a restriction enzyme made one cut in a plasmid and one cut in a piece of linear DNA, explain why the number of fragments that result differs.

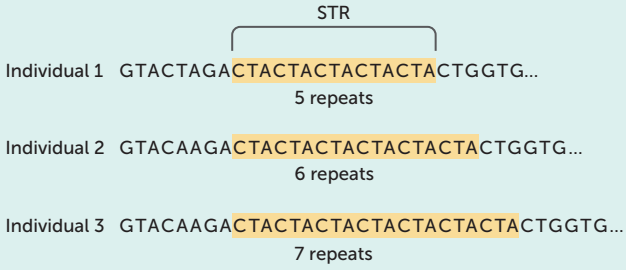
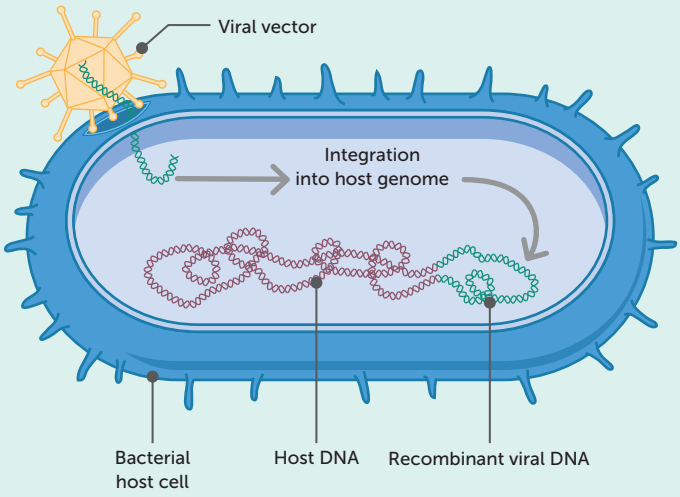
6.3 DNA TECHNIQUES AND VOCABULARY

DNA tools are used in conjunction with DNA techniques to help make products or solve biological problems. Techniques such as PCR and gel electrophoresis are used in multiple biotechnological processes, such as DNA sequencing, mapping, profiling and recombinant DNA methods. Microarrays will be discussed in addition to the main two techniques. Firstly, the language of biotechnology is needed to enable you to better understand the processes. The main terms are summarised in Table 6.4 (pages 175–177).

TABLE 6.4 DNA vocabulary used in DNA-based biotechnology

TERM	DEFINITION/DESCRIPTION	VISUAL AID
Amplify	To greatly increase the number of copies of a DNA sequence. This can be achieved, either in vivo by inserting the sequence into a cloning vector that replicates within a host cell, or in vitro by PCR.	 **FIGURE 6.11** Amplification
Annealing	Joins (hybridises) two pieces of DNA by complementary base pairing (joining of overhanging sticky ends). The two pieces are joined by weak hydrogen bonds only, and therefore temporarily. It is a biochemical process of bonding two segments of DNA at an optimal temperature of 50–60°C.	 **FIGURE 6.12** Annealing occurs between complementary base pairs.
DNA ladder	A collection of DNA fragments of known base pair lengths; a ladder is a standard or a set of molecular size markers used to compare the sample DNA with known DNA.	 **FIGURE 6.13** DNA marker (ladder)

TERM	DEFINITION/DESCRIPTION	VISUAL AID
Identification and extraction of target gene	Restriction enzymes can be used to identify a gene of interest. DNA fragments (genes) are cut out of their normal position in the chromosome. Automated DNA sequencing enables mapping of genes on chromosomes to help identify and extract target genes.	 **FIGURE 6.14** Isolating the gene of interest
Genome	All of an organism's genetic information, including all of the DNA that makes up the genes on chromosomes, mitochondria and (in the case of plants) chloroplasts. A genome is a complete set of instructions for making and operating an organism. Your genome contains the information (encoded in the DNA) to build your cells and your cells' components. It also carries the information necessary for the cells to function.	 **FIGURE 6.15** An organism's genome
Plasmid	A plasmid is an extra-chromosomal circular DNA molecule, distinct from the normal bacterial genome and non-essential for cell survival. It can replicate independently of the bacterial chromosome. Some plasmids are capable of integrating into a host genome. A number of artificially constructed plasmids are used as cloning vectors. They can be inserted as well as removed from bacteria. The loop is small and therefore contains only a few genes, but the genes are functional.	 **FIGURE 6.16** Plasmids found in a bacterium

TERM	DEFINITION/DESCRIPTION	VISUAL AID
Micropipette	A tool that dispenses small amounts of samples into PCR tubes or into the **wells** of an elecrophoresis gel. The volume is adjustable, and the tips are removed after every use to avoid contamination.	 Shutterstock.com/PHATCHARADA DUEANDAO **FIGURE 6.17** A micropipette
Short tandem repeat (STR)	STRs are sequences of DNA that repeat a certain number of times. They are highly variable segments of DNA, typical of non-coding and non-regulatory DNA, that occur throughout the genome and contain repeats of the same sequence of several nucleotides (e.g. CTACTACTACTA). The number of times the sequence is repeated is unique in different individuals.	 **FIGURE 6.18** STRs are unique to each individual
Vector	A vehicle that transports and introduces foreign DNA/genes into host cells. Using the host cell, the foreign DNA is reproduced in large quantities (cloned) and/or expressed. Vectors are often recombinant molecules containing DNA sequences from several sources.	 **FIGURE 6.19** A viral vector and a bacterial host cell

PCR: amplifying DNA

Each eukaryotic somatic cell generally has two copies of each gene, and prokaryotic cells have one copy. This small amount of DNA poses a problem for scientists wishing to work with it. Similarly, only a small sample of DNA may be available for analysis, for example, from a crime scene or in DNA samples obtained from bones. To increase the amount of DNA of a particular sequence, the biotechnologist has a technique to work with called PCR. PCR is a cyclic method used to rapidly amplify (replicate many times) relatively small numbers of particular sequences of DNA into extremely large numbers of copies. The DNA is then suitable for further laboratory uses such as gel electrophoresis and **DNA profiling**. PCR makes use of the enzyme taq DNA polymerase, which catalyses the formation of new DNA molecules from free nucleotides. Taq polymerase is used because of its ability to resist denaturing at high temperatures. This heat-stable polymerase was named after the bacterial species from which it was first isolated. The bacterial species *Thermus aquatics* lives in hot springs at temperatures of up to 95°C. Most enzymes would **denature** at this temperature, but taq polymerase remains stable.

A number of components are required for PCR: the DNA that is to be copied (template), the special DNA polymerase (taq polymerase), a buffer solution that contains salts and other chemicals to maintain the correct pH at which the polymerase can function, a supply of the four nucleotides (i.e. A, T, C, G, also known as deoxynucleotide triphosphates or dNTPs, from which to build the new DNA molecules), and two sets of single-stranded DNA primers. The primers are short, single-stranded DNA sequences (of around 20 nucleotides), chosen because they are complementary to the nucleotide sequences at either end of the DNA section that is to be copied. These are necessary as a starting sequence of nucleotides to which the DNA polymerase can begin to add new DNA nucleotides. DNA polymerase can only extend a DNA strand from an existing nucleotide; it cannot create a new complementary strand without primers to begin extending from.

The process of PCR involves a series of temperature cycles. Once this was carried out by moving tubes through various water baths, but it is now controlled automatically in machines known as **thermal cyclers**, or thermocyclers. Thermocyclers provide tight control over both the reaction temperature and the duration of each temperature step, ensuring efficient amplification. Within a few hours, billions of copies of the DNA sample can be made.

PCR has three steps (Figure 6.20).

1. Denaturation: The double-stranded DNA is heated to around 95°C, breaking the weak hydrogen bonds between the bases and thus causing the two template strands to denature (separate). The template strands have exposed nucleotides, ready for complementary base pairing, and will be used for the synthesis of the new strands.
2. Annealing: The temperature is reduced to 50–60°C, allowing the single-stranded DNA primers to anneal (join via hydrogen bonds) to complementary sequences on opposite ends of each strand. The DNA used is either genomic DNA or PCR products generated during the previous cycle. The reduced temperature is necessary to allow base pairing and the formation of hydrogen bonds.
3. Extension: The temperature is raised to 72°C, the optimum temperature for the DNA taq (heat-stable) polymerase used in PCR. Starting from the primers, new DNA strands are synthesised using DNA polymerase and the available nucleotides. At the end of this phase, there are two copies of each strand of DNA.

This cycle is repeated until sufficient quantities of the DNA are obtained to work with. Each cycle doubles the number of DNA strands; therefore, in just 20 cycles more than one million copies of the target DNA will be produced.

PCR is a process that amplifies a specific DNA sequence for analysis. The sequence of the primers determines the DNA sequence to be amplified.

Overview of PCR
Click on 'Application overview'.

PCR
View the interactive to learn more about PCR.

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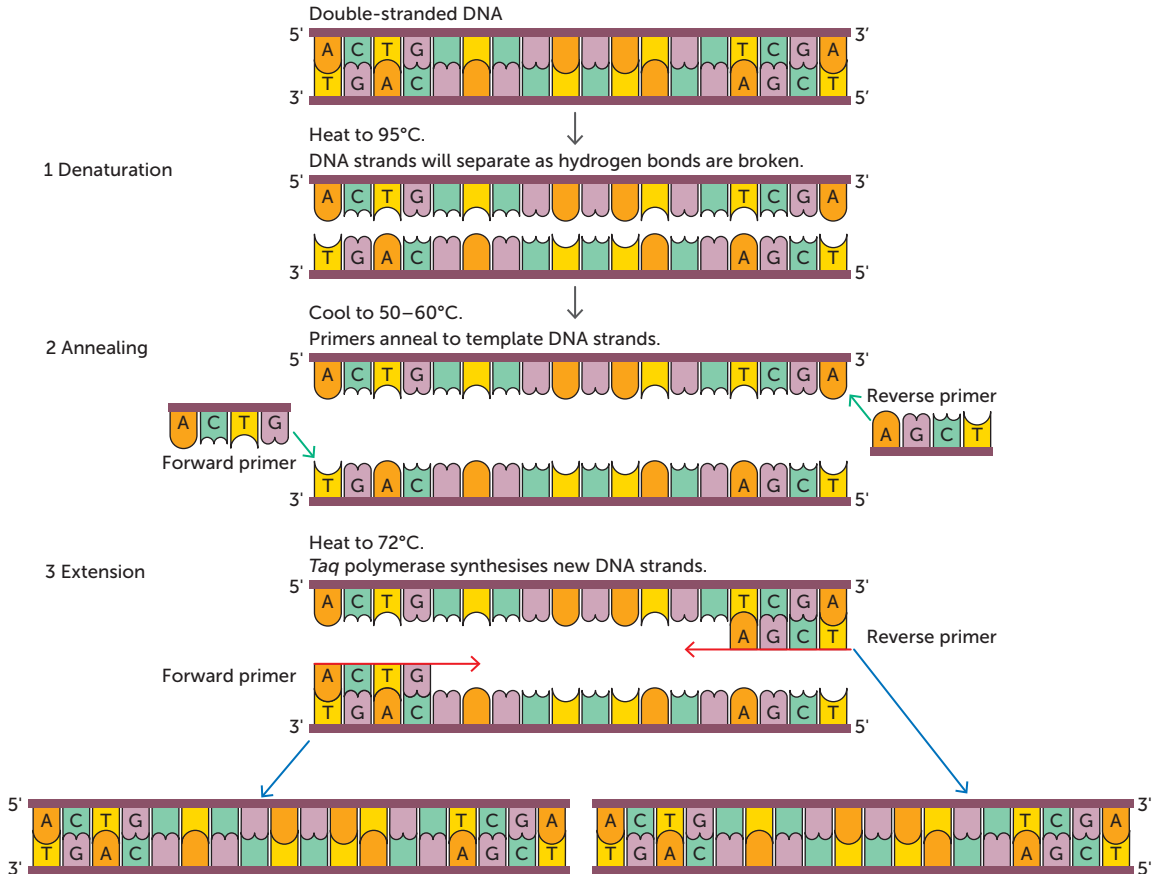

FIGURE 6.20 Amplifying DNA using PCR

Shutterstock.com/LJvGroup

FIGURE 6.21 A thermal cycler, in which PCR is carried out as an automated process

Shutterestock.com/Dantes Design

FIGURE 6.22 Thermal cyclers cycle through set temperatures.

TABLE 6.5 Summary of the PCR steps after the mixture is added to the thermal cycler

NAME OF STEP	TEMPERATURE OF MIXTURE	DESCRIPTION
Denaturation	95°C	The double-stranded DNA is heated to around 95°C, breaking the weak hydrogen bonds between the complementary bases, causing the two template strands to denature (separate).
Annealing	50–60°C	The temperature is reduced to 50–60°C, allowing the single-stranded DNA primers to anneal (join via hydrogen bonds) to complementary sequences on opposite ends of each strand. The primers attach according to complementary base-pairing rules to the 3' end of each template strand.
Extension	72°C	DNA taq polymerase extends the new strand, starting from the primers. New DNA strands are synthesised using taq polymerase and the available nucleotides. At the end of this phase, there are two copies of each original strand of the double-stranded DNA.
Repeat cycle	The process is repeated many times.	Each new strand can act as template strand; therefore, the DNA is amplified exponentially.

Question set 6.3a

REMEMBERING

1 Define:
   - **a** amplify
   - **b** anneal
   - **c** vector
   - **d** genome.

2 Describe STRs and how they are used in identification techniques.

3 Name the machine used for PCR reactions.

4 Name the main steps in PCR and the temperatures at which they happen.

5 State the components of a PCR reaction.

UNDERSTANDING

6 Describe the three steps of PCR.

APPLYING

7 If you start with five copies of a region of DNA, how many copies will be produced if your sample goes through 10 cycles of PCR?

Gel electrophoresis: visualising DNA

DNA molecules are far too small to see, but they have an overall negative charge due to the phosphate groups in the sugar–phosphate backbones, and this can be used to make their location visible.

Gel electrophoresis is a technique that can separate large, charged molecules (such as dyed fragments of DNA or proteins) according to size and charge, so that they can be visualised and identified by comparison with a standard. An **agarose gel** is melted and poured into a flat mould to cool. Wells are created by placing a plastic comb into the gel as it sets, creating indentations. The DNA samples and a DNA ladder are pipetted into separate wells in the row of wells. The medium commonly used for electrophoresis of proteins and nucleic acids is agarose gel because it allows the molecules and an electric current to flow through it.

The gel is placed in a tray filled with buffer solution, and positive and negative electrodes are attached at each end of the gel. When the electric current runs, the fragments are repelled by the negative electrode and move towards the positive electrode at the other end. The gel acts as a large sieve through which the DNA strands move under the influence of the electric current. Smaller strands can move through the gel matrix faster than the larger strands, which take longer to migrate through the gel. This method therefore separates DNA strands based on their size.

DNA itself will not be visible in the gel. To view the separated DNA fragments, **ethidium bromide** or another fluorescent DNA-binding dye is added to the agarose gel before it sets. The dye binds to the DNA and fluoresces under UV light at the completion of the investigation, showing a pattern of bands that can then be photographed. Each **band on the gel** contains many thousands of pieces of DNA of the same length. A band is a well-defined line in a **lane** on a gel. A lane is a corridor through which DNA passes after it leaves a well. The bands in each lane are compared with the bands in the standard. DNA fragments of the same length will overlap and be seen as one band.

Science Photo Library/Simon Fraser

FIGURE 6.23 A researcher pipetting genetic material into an agarose gel for electrophoresis

The position of bands on an agarose gel depends on the size of the DNA fragments in each band; the smaller the fragments, the further they move in a given time. To determine the size of a given piece of DNA, molecular biologists use a standard set of **molecular size markers** (the 'ladder'). These are pieces of DNA of a known number of base pairs (bp). By comparing the location along the gel of the DNA sample with that of the known molecular size markers, the size of the separated DNA fragments can be determined (Figure 6.24, page 182).

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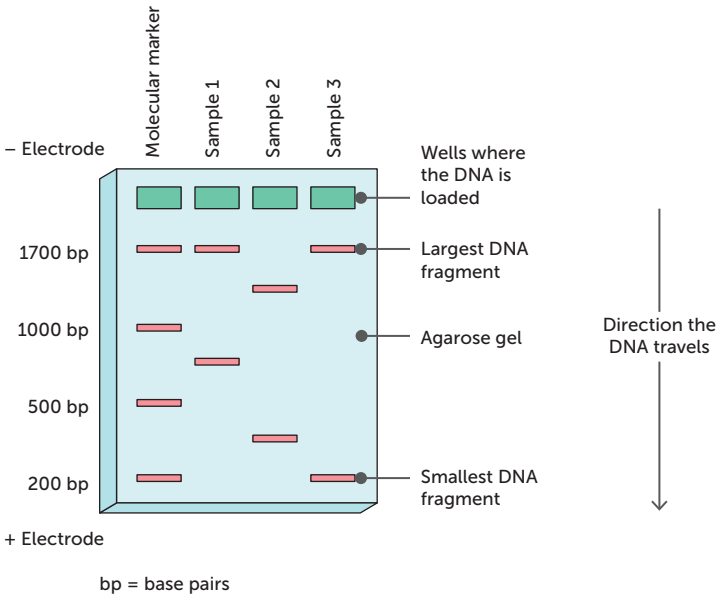
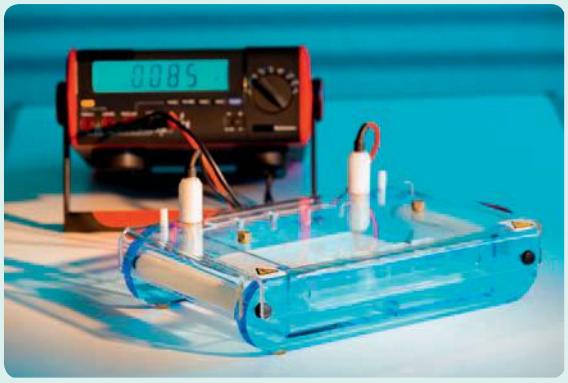
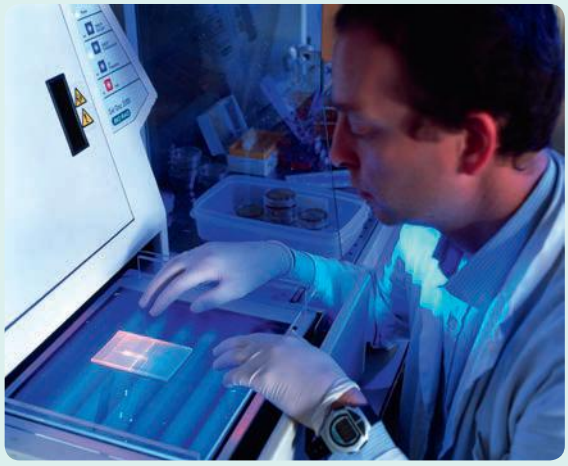


FIGURE 6.24 A set of molecular markers of known size (a ladder) is run alongside the samples and allows identification of the size of the DNA fragments migrating through the gel.

TABLE 6.6 Summary of the main steps of gel electrophoresis

NAME OF STEP	DESCRIPTION	VISUAL AID
Set up the apparatus	Set an agarose gel and make wells with a comb. Place the gel into the apparatus and pour a buffer solution over it for regulating pH. Cut the DNA fragments with restriction enzymes. Dye them with a binding chemical, such as ethidium bromide, which fluoresces under UV light.	 Shutterstock.com/Substance1productions **FIGURE 6.25** A comb is used to make wells.
Pipette samples	Pipette the samples and DNA ladder into the wells of the agar. Make sure the negatively charged samples are at the end of the apparatus where the negative electrode is situated. Discard used micropipette tips after each use.	 Shutterstock.com/Lab Photo **FIGURE 6.26** A micropipette is used to transfer the samples into the wells.

NAME OF STEP	DESCRIPTION	VISUAL AID
Turn current on	The negative molecules are repelled by the negative electrode and travel (migrate) towards the positive electrode, which they are attracted to. The smaller fragments move faster and further, and the larger molecules move more slowly and not so far.	 Shutterstock.com/science photo **FIGURE 6.27** The current is turned on.
Visualise and compare	Visualise the fragments by shining a UV light on the apparatus and photographing the results. The bands in each lane can be compared with the ladder to determine the length of the sample in bp.	 Science Photo Library/Mauro Fermariello **FIGURE 6.28** Visualisation of the bands may require UV light.
Hints for loading a gel	• Slowly lower the pipette. Don't stab the gel. Putting your tip too deep into a well or against the side of the well can result in puncturing the gel, allowing your sample to leak. • Use two hands on the pipette! You are aiming for a small target. Use your dominant hand to operate the pipette. Use your other hand to steady your pipette by placing a finger on the pipette shaft near where it meets the pipette tip. • Steady your arms by resting your elbows on the laboratory bench.	

Question set 6.3b

REMEMBERING

1 State the charge on a DNA molecule.
2 Describe the function, in gel electrophoresis, of the following:
   a agarose gel
   b primer
   c well
   d pipette.
3 Describe the process of gel electrophoresis.

UNDERSTANDING

4 Describe the purpose of gel electrophoresis.

5 Explain why a ladder is used when running an electrophoresis gel.

ANALYSING

6 Analyse the electrophoresis gel shown in Figure 6.29.

- **a** Which sample had the shortest fragment?
- **b** State the length of the fragment in sample A.
- **c** Are the wells situated near the negative or positive terminal?

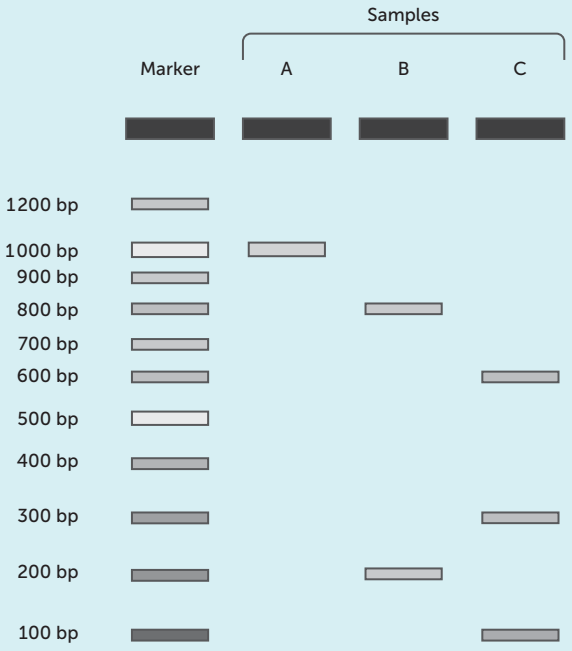


FIGURE 6.29 Results of a gel electrophoresis

Microarrays: probing for genes

Another identification tool used in biotechnology is called a microarray. A microarray is a collection of **gene probes** attached to a solid surface. A gene probe is a specific length of single-stranded DNA of between 20 and 40 nucleotides, or sometimes as large as 1000 nucleotides, that is complementary to a known sequence of DNA from a particular gene. A gene probe can measure the level of **gene expression** in a sample of DNA, and a microarray can screen a large number of genes at the same time. It is efficient and fast, identifying genes that are being expressed in certain individuals or breeds and also showing those genes that are not being expressed, for comparison. The tool can be used when scientists are trying to discern between genes that are desirable and genes that are not. For example, if some individuals are resistant to a disease, they may have a unique form of a gene that scientists would like to locate and analyse.

The DNA being investigated is heated to separate the two strands and expose their bases. The single-stranded probes then bind to any complementary sequences (Figure 6.30).

Gene probes have either a radioactive tag attached to them, which will show up when the microarray is exposed to photographic film, or a fluorescent dye tag, which shows up when the microarray is exposed to an ultraviolet light source. In the case of Huntington's disease, this technique can be used to determine which family members have the allele and will therefore develop the disease later in life.

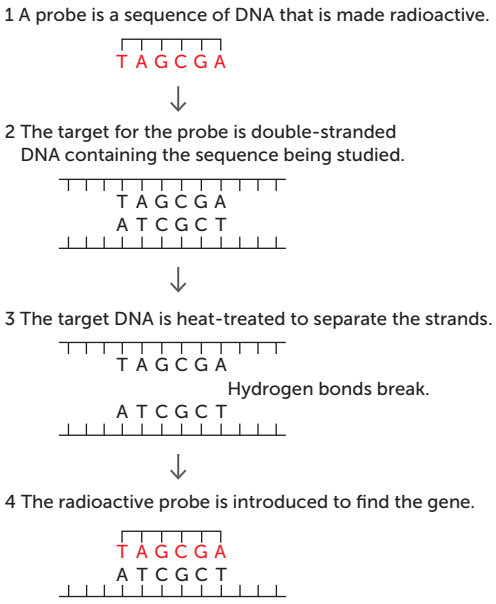


FIGURE 6.30 A probe is made up of 20–40 nucleotides complementary to its target sequence.

Most genes are present in the same quantity in every body cell – namely, one copy per haploid cell or two copies per diploid cell. However, only a small percentage of the genome is expressed in each cell, and the level at which a gene is expressed can vary widely.

Studying which genes are active and which are inactive in various cell types helps scientists to understand both how these cells function normally and how they are affected when various genes do not perform properly. In the past, scientists have only been able to conduct these genetic analyses on a few genes at once. With the development of DNA microarray technology, however, scientists can now determine the expression of thousands of genes at one time.

Gene probes can be natural nucleotide sequences, or they can be synthesised in the laboratory. They have a variety of uses, including finding a certain fragment of a gene after a sample has been separated by gel electrophoresis, identifying the position of a gene on a chromosome, and detecting an allele of a specific gene associated with a genetic disease.

Gene probing uses a single-stranded DNA molecule complementary to a gene of interest to identify, isolate or locate that gene on a chromosome.

A microarray consists of thousands of DNA probes arrayed on a single glass microscope slide or silicon chip (Figure 6.31). Each probe is designed to be complementary to a gene of interest in the target cell. The mRNA of the target cell is extracted, reverse transcribed into DNA (now called copy DNA, or cDNA) and labelled with a fluorescent marker. Fluorescently labelled DNA is then hybridised (allowed to bind) under stringent conditions to the probes on the slide. A scanner measures the fluorescence for each DNA probe on the slide. From this information, scientists can work out the activity of the genes in the cell: the stronger the fluorescence, the more mRNA in the original sample and therefore the greater the activity of each of the genes.

Microarray technology can be used to detect genetic diseases. For example, genes that are usually turned off in normal cells may be turned on, leading to uncontrolled cell division and cancer. Conversely, genes that suppress the development of tumours may be turned off. Microarray technology offers a way of diagnosing a cancer at the molecular level.

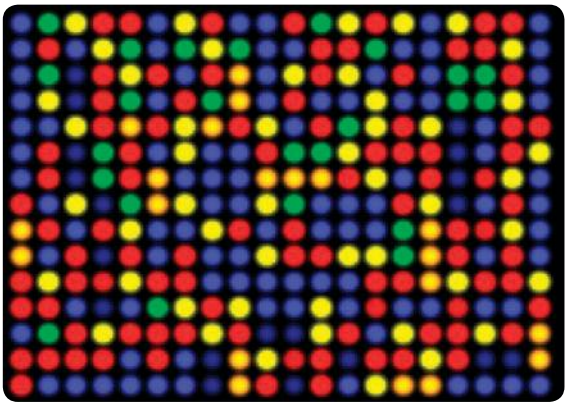


FIGURE 6.31 A DNA microarray indicating binding of cDNA to the DNA probes in one sample (red fluorescence), in another sample (green) and in both samples (yellow)

Key concept

Biotechnology techniques include PCR to amplify (increase) the amount of DNA, gel electrophoresis to visualise DNA, and microarrays to detect genes and their expression.

Question set 6.3c

REMEMBERING

1 What is a microarray?
2 Describe the purpose of a microarray.

UNDERSTANDING

3 Explain the meaning of hybridisation.
4 Explain how microarrays measure the level of gene expression.

APPLYING

5 Apply your knowledge of biotechnology tools and techniques to provide evidence for why microarrays are useful in medical diagnosis.

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6.4 DNA SEQUENCING

DNA sequencing refers to the methods and technologies used to determine the orders of the nucleotide bases [adenine (A), guanine (G), cytosine (C) and thymine (T)] in a DNA molecule. DNA sequencing enables us to perform a thorough analysis of the DNA because it provides us with very comprehensive information: the sequence of the nucleotides. Scientists cut DNA into fragments to sequence one section at a time. The entire set can then be put together to create a whole genome. The genomes of thousands of species have been sequenced, allowing genomes and genes to be compared. Knowing the sequences can help scientists determine the genetic code for particular phenotypes. There may be survival benefits in identifying, for example, genes that increase drought resistance or salt tolerance in plants. In addition, sequencing genes of different species has assisted scientists in determining genetic relatedness and evolutionary links.

DNA sequencing was originally done manually, using gel electrophoresis, and was called Sanger sequencing. It is now done using an automatic DNA sequencer that can sequence a large amount of DNA in a very short time. In this process, the four nucleotides are labelled with four differently coloured fluorescent dyes. As electrophoresis proceeds, a laser scans across the bottom of the gel, detecting the different dyes and consequently determining the base sequence. A computer can then automatically analyse the information from the gel to read the base sequence.

Faster and cheaper sequencing technologies are continually becoming available for use by biotechnologists. These methods are collectively called **next-generation sequencing (NGS)** techniques, and they use whole genomic DNA as a template, resulting in much greater sequencing efficiency. For example, one million DNA fragments of 700 bp can be sequenced in 24 hours, which is the equivalent of one full human genome every 5 days. Both Sanger and NGS apply the principles of complementary base pairing to determine the nucleotide sequence.

The first genome to be sequenced was that of a bacterial virus, and it was accomplished by Fred Sanger. The first species to have its genome sequenced was the bacterium *Haemophilus influenzae*, and it was done by Craig Venter in the 1980s. This was followed by the sequencing of larger genomes, such as those of the fruit fly, *Drosophila melanogaster*, and humans, *Homo sapiens*. The human genome took 13 years to sequence and was completed by 2003.

DNA sequencing can identify the exact nucleotide sequence of DNA fragments, which can then be used to determine the genetic basis for particular phenotypes.

The Sanger method: original, slower method

The Sanger method, also referred to as dideoxynucleotide sequencing or chain-termination sequencing, is based on the use of dideoxynucleotide triphosphates (ddNTPs) in addition to the normal nucleotide triphosphates (dNTPs) found in DNA. ddNTPs are essentially the same as nucleotides, except they contain a hydrogen group (H) on the 3' carbon instead of a hydroxyl group (OH). These modified nucleotides, when integrated into a DNA sequence, prevent the addition of further nucleotides, thus stopping the elongation of the DNA chain. This occurs because a phosphodiester bond cannot form between the dideoxynucleotide and the next incoming nucleotide, and thus the DNA chain is terminated.

Although the various reagents, equipment and strategies for carrying out DNA sequencing have changed to improve the simplicity, speed and reliability of the process, the basic procedure has not changed over the decades since its invention.

The following steps form the basis of the Sanger method:

1. The region of DNA to be sequenced is identified, cut and amplified (using a tool such as PCR) and then heated and denatured to produce single-stranded template DNA.
2. Template DNA, primer, DNA polymerase, all four types of dNTPS (A,T,C,G) and one type of dyed ddNTP are added into the reaction mixture.

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3 The sequencing DNA primer is annealed to the single-stranded DNA at the 3' end of the original strand, which provides a starting sequence for synthesis.
4 DNA polymerase extends the new strand by attaching complementary dNTPs in the 5' to 3' direction.
5 When a dideoxynucleotide that has been coloured with fluorescent dye attaches randomly, the newly synthesised strand terminates (the ddNTP prevents the formation of a phosphodiester bond).
6 By performing four separate reactions, four separate sets of chain-terminated fragments are produced.
7 Following the termination step, heating to denature the partially double-stranded molecules releases the single-stranded chain termination molecules of the various lengths from their templates.
8 They can then be separated using gel electrophoresis. The nucleotides, which are differently coloured, run in separate lanes.
9 As gel electrophoresis proceeds, a laser scans across the bottom of the gel, detecting the different dyes and revealing the base sequence. The terminated strands line up from smallest to largest. The various colours enable identification of the nucleotide in each position.
10 The sequence of the original region of DNA is then finally deduced by examining the relative positions of the dideoxynucleotide chain termination products in the four lanes of the denaturing gel.

DNA sequencing interactive and animation
View these resources to further your understanding of Sanger sequencing.

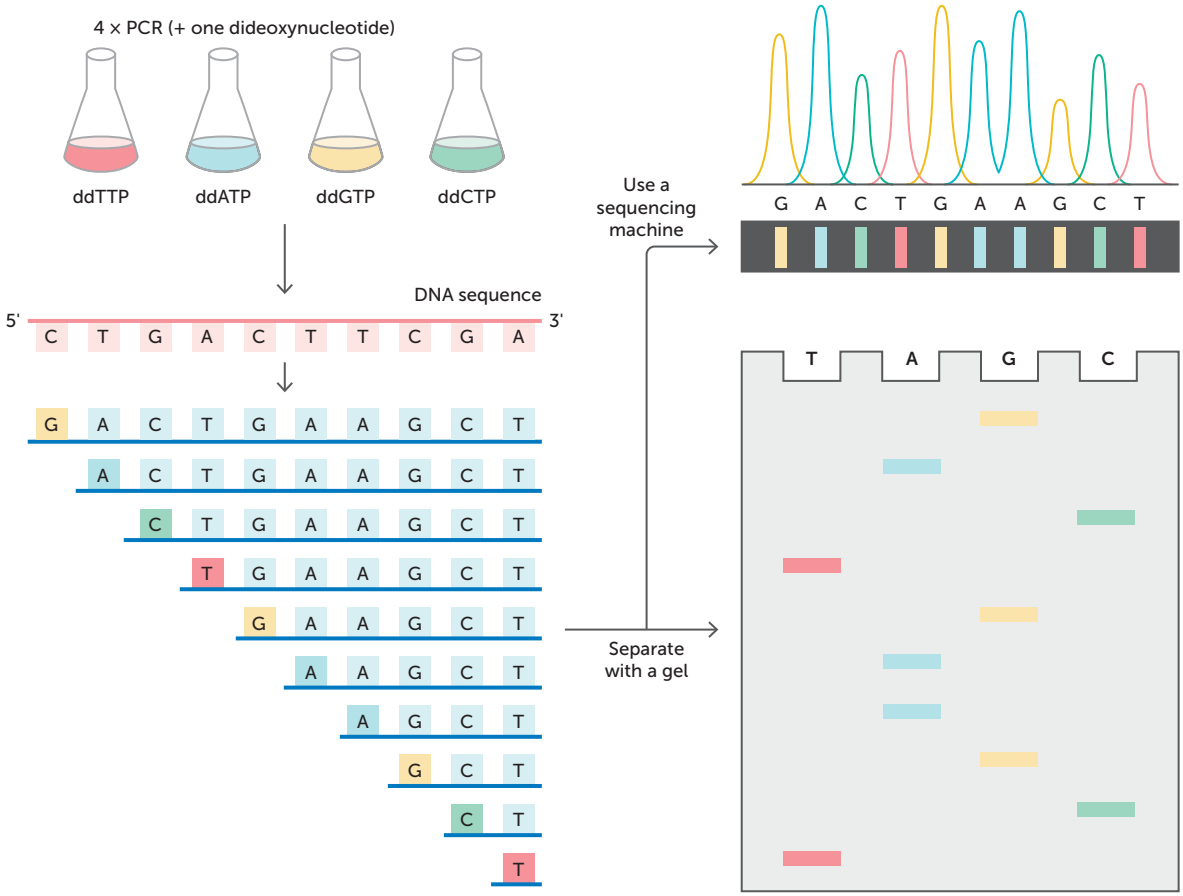

FIGURE 6.32 The Sanger sequencing method. The terminated nucleotides are separated and then lined up to produce a sequence.

Next-generation sequencing: a new, more efficient method

NGS applies the same principles as the Sanger method, but the technology is more advanced.

The basic steps are DNA preparation, sequencing and analysing.

1. DNA preparation

   DNA is isolated and purified, then cut into fragments of approximately 300 bp in length. The fragments are then amplified using a PCR-type method to create massive numbers of identical copies. The resulting fragments are single stranded. The different types of fragments are placed into unique wells and barcoded.

2. Sequencing

   The multi-well plate contains the assorted fragments. In each well, modified versions of the four types of DNA nucleotides wash over the mixture. The nucleotides hydrogen bond to the DNA template strand according to complementary base-pairing rules. Each nucleotide has one of four fluorescent tags attached. The tags indicate the positions and thus the order of the four nucleotides in the sequence being analysed. A terminating set of nucleotides is also in the mix, which prevents further elongation of the new strand. After copying the forward DNA strand, the reverse strand is similarly processed. Each time a chemically tagged nucleotide attaches to the template strand, there is a flash of light and this is recorded. A different colour of light flashes for each different type of nucleotide added.

3. Data analysis

   The recorded light flashes reveal the sequence of nucleotides of the template strand in each well. The sequencing software identifies the nucleotides by the order of the colours recorded.

In both NGS and Sanger sequencing, DNA polymerase adds fluorescent nucleotides one by one onto a growing DNA template strand. Each incorporated nucleotide is identified by its fluorescent tag. One colour represents one nucleotide. The major difference between Sanger sequencing and NGS is the number of fragments that can be sequenced at once: one versus many. The Sanger method only sequences a single DNA fragment at a time. In contrast, NGS sequences millions of fragments simultaneously per run. NGS can sequence hundreds to thousands of genes at one time.

Genome sequencing is mostly used in medicine to identify abnormal gene function via DNA microarrays. However, with the more rapid sequencing techniques, the applications are widening to include conservation and agriculture.

6.1 APPLICATION

Comparative genomics between humans and fruit flies

Comparative genomics involves the comparison of different genomes. Recent studies have discovered that humans share 60% of their genes with fruit flies, *Drosophila melanogaster*. Two-thirds of the genes involved in human cancer have equivalent genes in the fruit fly genome. The evolutionary links between species, or their genetic relatedness, can also be determined by comparing genomes. Humans and fruit flies diverged from each other about 990 million years ago (mya), but we only diverged from chimpanzees about 5 mya.

CASE STUDY

Use of next-generation sequencing to study ecosystem biodiversity

Professor Michael Bunce is a molecular biologist at Curtin University in Perth, Australia. He uses NGS to analyse environmental DNA (eDNA). eDNA is the DNA that organisms shed into their immediate surroundings, usually their habitat. Michael Bunce's team extract, amplify (using PCR) and analyse degraded DNA. Using a combination of DNA barcoding and NGS, samples of DNA that in the past were too

small or degraded to analyse can now be analysed successfully.

The team's first published paper showed that DNA could be extracted from prehistoric Siberian permafrost cores and New Zealand cave sediments, then sequenced successfully. This was landmark work, because it demonstrated that we don't need fossils to identify the animals that were present in an area many centuries ago. We can detect the DNA that these animals left behind and use the data it provides to study how plants and animals have changed over time. Prior to NGS, Michael's team used the slower Sanger sequencing technique. They had to extract DNA from the sample, clone it into bacteria, and then sequence the bacterial plasmids one bacterium at a time. It was time consuming, costly, and didn't delve deeply enough into the species present in the sample.

Thanks to NGS, the team can more accurately and rapidly sequence eDNA. This means that, from prehistoric DNA, they can determine what species lived where. They have been able to concentrate their attention on genes that have evolved and genes that have been conserved over time. One method they use is to concentrate a litre of seawater down, leaving its organic components, from which they can extract the DNA. This method yields information about the biodiversity in each sample. For example, it is possible to design PCR assays that use 'molecular magnets' able to latch onto certain sequences of fish DNA to study what fish have been in a sample of seawater. A species list can then be created. The presence or absence of various taxa can be used to build a holistic picture of the biota in a specific environment and how it interacts.

Recent studies by Michael's team have assessed biodiversity in marine samples and evaluated rehabilitation success in restoring native ecosystems after mining or oil exploration. They have used these eDNA analysis approaches on ice cores, dirt from swamps, archaeological sites, seawater, faecal samples and herbal medicines.

Courtesy of Curtin University

FIGURE 6.33 Professor Michael Bunce from Curtin University

Courtesy of Curtin University

FIGURE 6.34 Taking environmental DNA samples at Rottnest Island

Currently, in collaboration with CSIRO, Michael's team have been analysing samples collected off the coast of Rottnest Island, near Perth. They have been successfully isolating and sequencing eDNA, seeking insights into past ecosystems and endeavouring to make predictions about future marine life in relation to our changing climate. In addition, marine environmental DNA biomonitoring has revealed seasonal patterns in biodiversity, and identified ecosystem responses to unusual climatic events. Michael is hoping to get high school students involved in this monitoring program.

Questions

1. Describe what eDNA is.
2. Explain how the sequencing of eDNA can help scientists predict ecosystem changes in the future.

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Question set 6.4a

REMEMBERING

1 State the purpose of DNA sequencing.
2 Recall the steps of Sanger sequencing.
3 Name the species that was the first to have its whole genome sequenced.

UNDERSTANDING

4 Describe the main differences between Sanger sequencing and NGS.
5 Explain what the flashing lights during NGS represent.

DNA sequencing enables mapping of species' genomes

A genome includes the entire set of genetic instructions, both the genes and non-coding sequences, of an organism. DNA sequencing has enabled the mapping of genomes. **Genetic mapping** involves identifying and recording the positions (or relative positions) of genes on chromosomes. When a species' genome is mapped, all of the chromosomes in a somatic cell are mapped. Once the position of a gene, known as its locus, is known, it can be shown on a diagram.

A **genetic marker** ('landmark') is a nucleotide sequence that is associated with a specific trait. Genetic markers may include short DNA sequences, such as STRs, or longer sequences, such as genes. Genomics is the study of entire genomes, including the complete nucleotide sequence and organisation, and the variation in the sequence both between individuals and between species. Variations in the sequence can be identified as being associated with identifiable disorders or beneficial phenotypes.

Working out the genetic map is complex. Gene mapping is supported by the theory of crossing over of chromosome segments during meiosis. When small sections of chromosomes are swapped, genes that are located close to one another are likely to be swapped at the same time. As a result, those genes would be inherited together. Two genes that are on the same chromosome are said to be 'linked', and the distance between those genes is called the 'linkage distance'. The smaller the distance, the more likely it is that the two genes will be inherited together.

If several generations of offspring inherit the same disease (or beneficial trait, such as salt tolerance) and the same DNA marker, it is probable that there is a gene with an allele associated with the disease (or beneficial trait) located near the marker. That kind of information can enable scientists to map a gene for a particular phenotype at a cytogenetic location (relative position) on a chromosome. The cytogenetic location is illustrated by using the distinctive patterns of bands created when chromosomes are stained with certain chemicals.

DNA tools can be used to extract DNA fragments from samples and to detect subtle differences in DNA patterns (such as in STRs) that help distinguish one individual from others of the same species. Individuals with a disease or trait of interest may have a slight variation in a DNA pattern compared with other individuals unaffected by that disease or trait. DNA markers of this type don't, by themselves, identify the gene responsible for the disease or trait. They may, however, indicate to researchers that an allele associated with the disease or trait is present and approximately where the relevant gene is located on the chromosome carrying that marker.

Another type of mapping, called **physical mapping**, determines the precise molecular location of the genes on a chromosome. The molecular location is based on the sequence of the nucleotides. Physical mapping can be used to improve the accuracy of genetic mapping. Scientists may be able to calculate the physical distance in base pairs (bp) between known DNA sequences, such as genes, by working out how many base pairs are between them. Physical mapping can also indicate the size of genes. Both genetic maps and physical maps are required for building a complete picture of the genome.

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Sequencing enables mapping, and mapping enables sequencing

One strategy used is to build a genetic map by sequencing a genome. Sequenced DNA fragments (parts of the genome) can be pieced together by comparing them with the growing genome sequence. The loci of some genes may then be found and they can then be added to genetic maps. On the other hand, the map may help form the framework for sequencing the genome. Figure 6.35 is a genetic map of the chromosomes of a fruit fly. The distance between genes and the reference position 0.0 is shown on the left of each chromosome and the associated phenotypes are on the right. Genetic markers, linkage mapping and physical mapping are all used in genetic mapping and building our understanding of the genome.

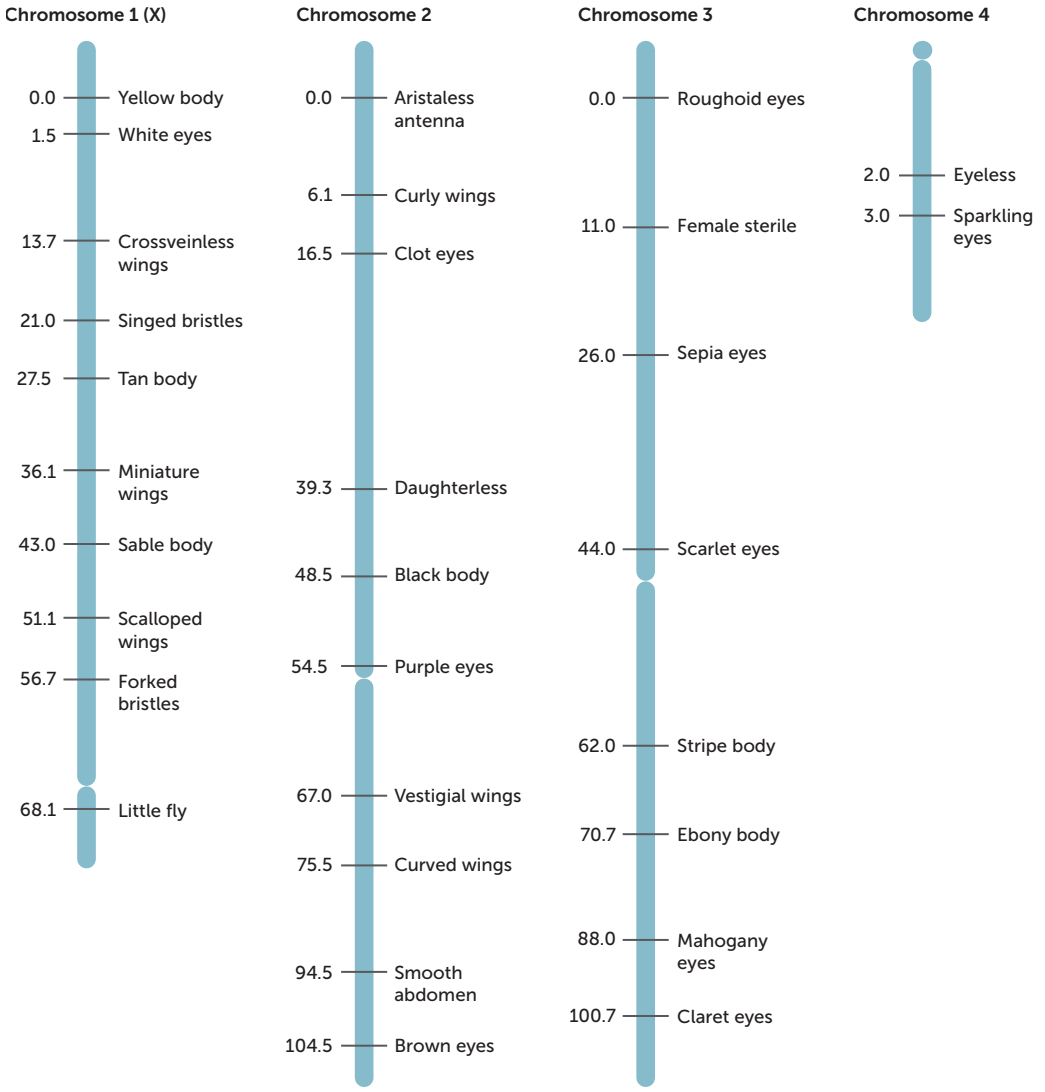

FIGURE 6.35 A genetic map of the chromosomes of the fruit fly, *Drosophila melanogaster*

Question set 6.4b

REMEMBERING

1. Define genome map.
2. Describe a genetic marker.
3. State the purpose of a genetic marker in gene mapping.

UNDERSTANDING

4. Explain how DNA sequencing can enable genome mapping.
5. Describe the role of a genetic (linkage) map.

SCIENTIFIC LITERACY

Using genetic markers in the quest to save our vulnerable burrowing bettong

Nationwide, the population of burrowing bettongs (*Bettongia lesueur*) is estimated at 19 000 individuals, although their population does fluctuate with rainfall. In WA their conservation status is deemed 'conservation dependent'. The decline in numbers of burrowing bettongs on the mainland appears to be largely due to predation by feral cats and foxes. Conservation of burrowing bettongs is reliant upon the establishment of feral predator-free areas on the mainland and on preventing cats and foxes from establishing populations on islands. The Australian Wildlife Conservancy is working with the Shark Bay Marsupials Recovery Team to support their conservation in the field.

Meanwhile, the School of Biological Sciences at the University of Western Australia are working with DNA to support the bettong conservation efforts. Genetic variation in the bettong is decreasing because only small populations remain. Their isolation prevents interbreeding between populations. Some reasons for the loss in genetic variation are inbreeding and genetic drift (random loss of alleles over generations).

Scientists recently published a report about a population genomics study. To increase genetic variation in the bettong, the scientists facilitated the mating of two bettong subspecies, a strategy known as genetic admixing. Genetic admixture is the introduction of DNA, through an individual from a distantly related population or species, as a result of interbreeding between populations or species that have been reproductively isolated and become genetically differentiated. Admixture results in the introduction of new genetic lineages into a population.

Using high-resolution genomic markers through double-digest restriction site–associated DNA sequencing (ddRAD-seq) and life history data collected over 9 years of monitoring, this study investigated the genetic and fitness consequences of admixing two genetically distinct subspecies of *Bettongia lesueur* in a conservation translocation. The study supported the hypothesis that mixing multiple source populations would be beneficial in the conservation of the threatened species.

The introduction of individuals into inbred populations can provide a 'genetic rescue' effect by infusing new genetic variation and relieving the deleterious effects of inbreeding, leading to improved fitness and adaptive potential. This is an example of admixture being used to improve conservation outcomes for threatened species, and this technique has been documented in a range of mammals, including the mountain pygmy possum.

Genomic DNA was extracted from the biopsied ear tissue of burrowing bettongs, sequenced and studied with the use of genetic markers. Individuals from two subspecies were mated and any outbreeding depression (subsequent loss of fitness) was assessed. The admixed population showed significant increases in all genetic diversity parameters compared with either founder population. Using high-resolution genomic markers through ddRAD sequencing, this study showed that the two founder populations were genetically distinct and readily interbred, resulting in an increase in genetic diversity, with no negative effects on survivorship or reproductive capacity.

Nature Picture Library/Jiri Lochman

FIGURE 6.36 The burrowing bettong's survival is under threat.

Questions

1. State the conservation status of the burrowing bettong in WA.
2. Describe how workers in the field and workers in the laboratory are helping in the fight to conserve these animals.
3. How were genetic markers used in this study?

6.5 DNA PROFILING

DNA profiling (also known as DNA fingerprinting) is a technique used by scientists to identify an individual by comparing an unknown sample of DNA with known DNA profiles. The scientist looks for a match within the non-coding regions of an individual's genome. Non-coding regions of DNA have satellite DNA – long stretches of DNA made of repeating units called short tandem repeats (STRs). Typically, individuals, even of the same species, possess unique banding patterns of DNA that make up their STRs. This reflects their unique genetic information. The banding patterns are visualised by gel electrophoresis and compared with known DNA profiles to distinguish between individuals.

STRs are sections of non-coding DNA that are repeated many times. The repeating units of STRs are 2–5 bases in length (e.g. AGAGAGAG). Larger sequences that are repeated are called **variable nucleotide tandem repeats (VNTRs)** and are greater than five bases in length. These are genetic markers that are highly variable from individual to individual. For example, one organism may have the sequence ACAT repeated 12 times, whereas another organism of the same species may have 18 repeats. The repeated sequence can be cut using restriction enzymes, amplified using PCR, and fluorescently tagged. Its length (number of repeats) can be determined, and it can be visualised using gel electrophoresis.

A summary of the process of DNA profiling follows.

1. DNA fingerprinting starts with isolating a DNA sample from any somatic (body) cell. A specific fragment is cut at a recognition site using restriction enzymes.
2. PCR makes many copies of the small amount of DNA.
3. The fragments can be separated, the length of the fragments visualised and the number of repeats determined by the use of gel electrophoresis. Smaller fragments have fewer STRs. They migrate further during electrophoresis.
4. The DNA is visualised under a UV light.
5. The profile is the unique set of patterns of bands. The patterns of bands are different because we are all genetically different and unique (other than identical individuals from multiple births).

Fuse school: DNA fingerprinting
Watch this video to further your understanding of DNA fingerprinting.

The STR and VNTR repeats are present in all members of the population, but the number of the repeats varies between individuals (Figure 6.37). Each individual usually has two alleles for each STR, one from each homologous chromosome. DNA profiling identifies people based on differences in the length of their DNA repeats for a large number of individual STRs. As every individual has their own unique numbers of repeats, this forms the basis of identification.

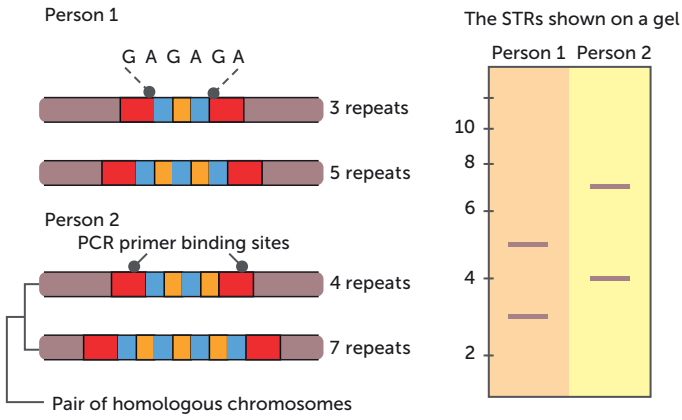

FIGURE 6.37 STRs vary between individuals.

6.2 APPLICATION

Superb fairy-wrens – not as faithful as you thought!

DNA profiling can be used to determine paternity. Through the use of microsatellite markers, a type of STR, researchers have established that approximately three in four superb fairy-wren (*Malurus cyaneus*) chicks are sired by a male other than their social fathers. This has come as a surprise, because females have never been seen copulating with males other than their partners. It is believed that all copulations with other males take place under the cover of darkness, either early in the morning or late in the evening.

Dreamstime/Ben Twist

FIGURE 6.38 Paternity testing shows that superb fairy-wrens are promiscuous.

Questions

1 What is paternity testing?
2 Describe three different uses of paternity testing.
3 Are there any disadvantages in using paternity testing?

Key concept

DNA sequencing and profiling use the tools and techniques of biotechnology to study a species' complete genome and identify individuals within a species.

Question set 6.5

REMEMBERING

1 What does the acronym 'STR' stand for?
2 List three genetic tools or techniques used to build an individual's DNA profile.

UNDERSTANDING

3 Describe the role of PCR and gel electrophoresis in DNA profiling.
4 Explain how DNA profiles enable scientists to distinguish between two individuals. Describe the factor that makes them unique.

ANALYSING

5 Analyse the image below to work out which DNA profile (labelled 1–3) matches the STRs found in the individual's DNA.

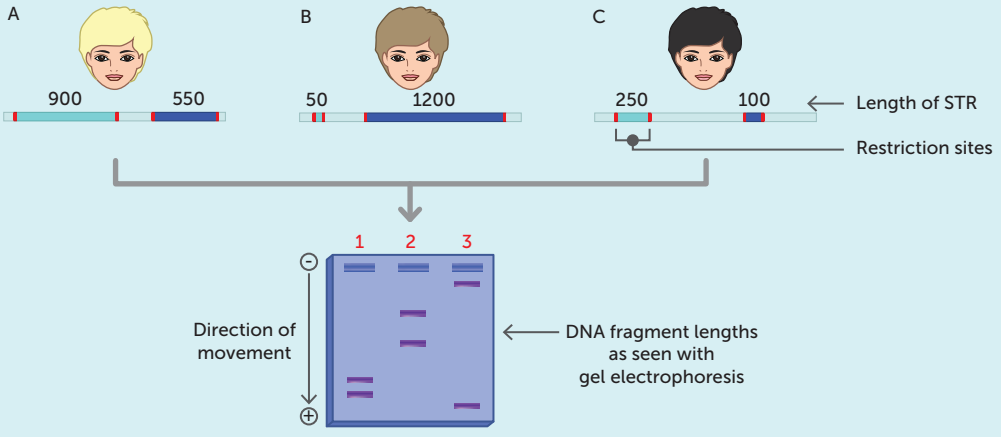

FIGURE 6.39 DNA profiles of three individuals

6.6 RECOMBINANT DNA TECHNOLOGY AND TRANSGENIC ORGANISMS

Recombinant DNA is DNA that is composed of one or more genes from two different organisms, usually two different species. 'Foreign' DNA is transferred into the genome of the host organism and is then expressed in the host. The host organism is known as a **transgenic organism** or a **genetically modified organism (GMO)**. The introduced gene instructs the transgenic organism to produce the desired trait through gene expression. The trait may be passed on to future generations. Recombinant DNA technology is widely used in agriculture, environmental conservation and medicine. Many careers that require this technology include agricultural, environmental, medical, veterinary and forensic science.

Recombinant DNA technology includes the use of DNA tools and techniques including restriction enzymes, ligases, vectors and cloning. A vector can be introduced into a host organism, which is grown to produce multiple copies of an incorporated DNA fragment in culture (**gene cloning**). Next, clones containing a relevant DNA fragment may be selected and harvested. This process involves the insertion of DNA fragments that have a desirable gene sequence (from a variety of sources) into a target organism via an appropriate vector.

Many scientists work on transforming organisms into transgenic organisms. Transgenic organisms have been engineered for a variety of desirable traits, including disease-resistance, faster growth rate, greater product quality and yield, and tolerance to adverse environmental conditions. For example, a sheep may be transformed with a gene for the blood clotting factor IX so that this protein is secreted in its milk. Factor IX can then be harvested from the milk and used to treat people with certain forms of haemophilia. For genes to be inserted into complex animals and plants, a method is needed for delivering the gene to the organism's cells. The gene needs to be able to function when it arrives in the host organism, whether it is a plant or an animal.

Plasmids are a common tool used by scientists to produce recombinant bacteria. Plasmids have been highly engineered as vectors for molecular cloning and for the subsequent large-scale production of important molecules, such as insulin. A valuable characteristic of plasmid vectors is the ease with which a foreign DNA fragment can be introduced. The same principles apply when plasmids are used as vectors for larger host organisms. Several human proteins are expressed in the milk of transgenic sheep and goats, and some are expressed in the eggs of chickens. Staples like corn, potatoes and tomatoes were the first crop plants to be genetically engineered.

Plasmids are used to insert DNA into bacteria. A plasmid is a circular piece of DNA that is found in bacteria and which reproduces independently of the bacterial chromosome. The key to using plasmids as DNA copiers lies in our ability to incorporate foreign genes into plasmid DNA and in their ability to replicate in bacteria. A number of steps are involved in this process (Table 6.7, pages 196–197). An alternative to using PCR to generate a large number of copies of a DNA sequence is to insert it into bacteria. This process is called gene cloning and it has multiple advantages. It allows replication of larger segments of DNA and permits the analysis of any gene, and its associated proteins, including those of DNA sequences from the environment where the organisms are living and active.

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TABLE 6.7 Summary of the technique used to create a transgenic organism (GMO) using a plasmid

STEP	DESCRIPTION	VISUAL AID
Identify and isolate the desired gene	The use of DNA sequencing or mapping may help scientists locate a desired gene.	
Extract using restriction enzymes	To cut the gene of interest out of a donor organism, an enzyme that cuts at a specific recognition site is used. (It does not cut at a random site but only at the sequence of nucleotides it codes for.)	
Use the same restriction enzymes to cut the plasmid/ vector	Plasmids are extracted from bacteria by rupturing the cell walls. Then the plasmid is cut open using the same restriction enzyme that was used for the gene to be inserted, so that both pieces of DNA have complementary sticky ends.	
Annealing and ligation	Weak attractive forces (hydrogen bonds) draw the complementary nucleotides together. This is annealing. Then DNA ligase binds the foreign DNA fragment into the plasmid DNA ('ligation') by catalysing the formation of two covalent phosphodiester bonds between the 3' hydroxyl group of one nucleotide and the 5' phosphate group of another. After binding, the DNA fragment becomes a permanent part of the **recombinant plasmid**.	
Place recombinant plasmid into bacteria for cloning	The recombinant plasmids function as vectors as they are added to a bacterial culture. They are taken up by some bacteria, in which they replicate many times. This is known as gene cloning. In the normal process of growth and division, bacteria replicate the plasmid via binary fission, and thus numerous copies of the incorporated foreign DNA are made.	
Transformation and expression	The process of bacteria taking up the plasmid (containing foreign DNA) and incorporating the desired gene into its genome is called transformation. If the gene is expressed, it has been transcribed and translated into a protein that may be used by the host organism.	

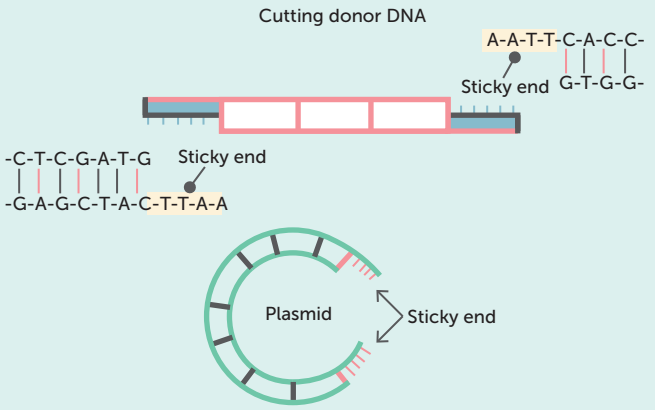


FIGURE 6.40 The same restriction enzyme is used to cut the donor DNA and the plasmid DNA.

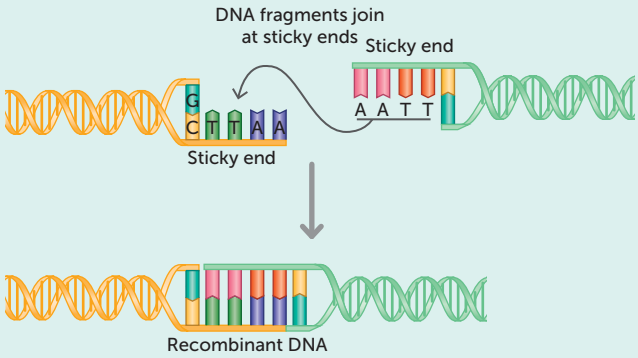


FIGURE 6.41 Annealing occurs between the complementary sticky ends, and ligation joins the sugar–phosphate backbone between the two fragments.

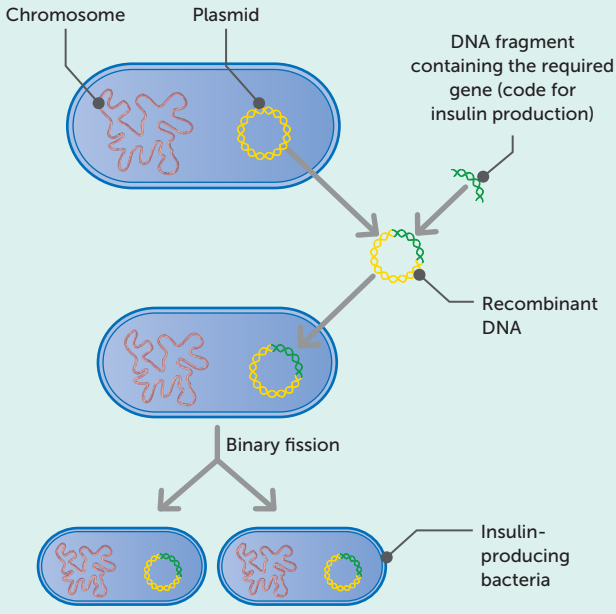


FIGURE 6.42 Transformation of bacteria and gene expression

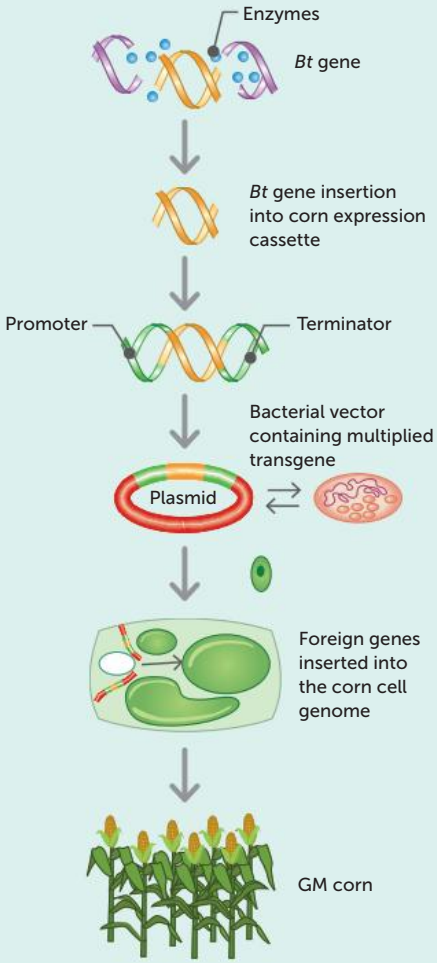
STEP	DESCRIPTION	VISUAL AID
Vector for another host organism	To make a transgenic species, the bacterial vector is inserted into a host organism by a gene gun or other method, such as mixing it with embryos. An example of this is Bt corn – a recombinant corn species that contains the *Bt* gene for insect resistance.	
New phenotype observed	The desired gene expression is the phenotype that was originally observed in the donating organism. For example, Bt corn has a new phenotype of resistance to cotton bollworm. The desired gene was found in a Bt bacterial cell.	

FIGURE 6.43 Bacterial vectors can deliver the target gene into a host.

Vectors: transferring genes

A number of different methods, including gene guns with gold particles coated in DNA, have been used to deliver genes. In most cases, the gene is inserted into a vector that will carry the gene to the target organism. In this context, a vector is a tool that can be used to transport DNA from one organism to a recipient host. Plasmids and viruses can act as vectors because they can transport small sections of DNA from one organism to another.

Plasmid vectors

Agrobacterium tumefaciens is a bacterium that acts like a vector in nature by transferring genes found on its plasmids to other organisms. It is commonly used in recombinant DNA technology. Plasmids can be copied numerous times, regardless of whether the bacterial host is replicating its own DNA, and every time a plasmid vector is replicated, so is the introduced DNA that it contains. Purified recombinant plasmids can be inserted into a new organism directly. However, despite showing great potential, this method is not currently an efficient method of gene delivery, because plasmid DNA is not very stable in body cells in this form.

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Viral vectors

Mechanism of recombination (genetic engineering)
Watch the animation then answer the questions below.

Viruses infect target cells by injecting their nucleic acid into the host cell. By using recombinant DNA techniques, it is possible to insert desired genes into viral DNA or RNA, and use the virus to insert this new gene into the target cells. Viruses can accept large DNA inserts, making it relatively easy for them to accept foreign genes. As viruses are pathogens, it is also necessary to remove or disable the genes in the virus that cause disease symptoms. Two types of virus currently being used in this way are the adenovirus and the retrovirus (Figure 6.44). The main problem with using viruses as vectors is that human immune systems attack viruses, and this may decrease their chance of survival within their new host. Furthermore, viral DNA insertion in the host genome can sometimes disrupt normal gene regulation and result in the development of cancer.

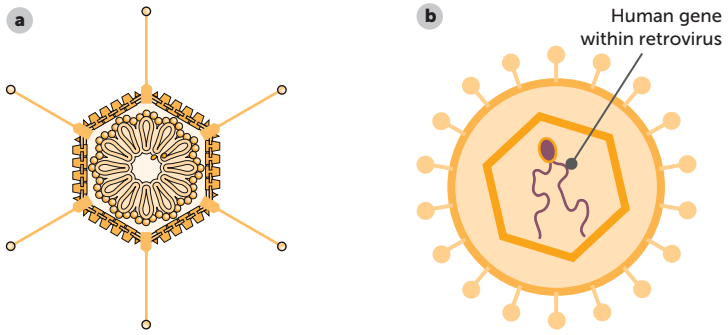

FIGURE 6.44 A vector can be **a** an adenovirus or **b** a retrovirus.

Key concept

Recombinant DNA technology uses the tools and techniques of biotechnology to create transgenic organisms (combining foreign DNA with host DNA). Vectors can be used as transgenic organisms to transfer genes into another organism.

Question set 6.6

REMEMBERING

1. Define transgenic organism.
2. Define vector.
3. List some desirable traits that inspire scientists to create transgenic organisms.

UNDERSTANDING

4. Draw a labelled and annotated diagram to show the sequence of events that results in the formation of recombinant DNA.

   Annotations to include:
   - A gene of interest is isolated and cut out with a restriction enzyme, and a plasmid is cut open with the same restriction enzyme.
   - A gene is inserted into a plasmid, usually by joining sticky ends and complementary base pairing (annealing).
   - The sticky ends are sealed together by DNA ligase.
   - The recombinant plasmids are cloned and many copies produced OR the recombinant plasmids are inserted into new host cells (via a virus, a bacteriophage or a bacterial vector) by shooting, spraying, microencapsulation or heat treatment.
   - A gene is expressed in a recombinant organism.

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CHAPTER 6 ACTIVITY AND INVESTIGATIONS

6.1 ACTIVITY

Brood parasitism and family size in black swans

Family size in black swans usually varies between one and seven. Interestingly, family size distribution seems to be bimodal, with some families containing 1–3 cygnets, but others containing up to about 14 cygnets. This has led many to speculate that the larger families are the result of brood parasitism – a female laying her eggs in the nest of a second female and leaving this second female to raise her young. This process is quite common in ducks but has not been investigated thus far in black swans.

One way to determine whether a female is the biological mother of her cygnet is to create a DNA profile for both the mother and the cygnet and determine whether the cygnet shares half of the mother's profile.

Aim

To determine, using DNA profiling, whether brood parasitism occurs in black swans and whether this might explain the larger number of cygnets in some families

You will need

Each student will require a ruler.

What to do

Consider the DNA profile in Figure 6.50 (page 212). The necessary DNA was obtained by capturing swans, collecting a small blood sample from each, and extracting the DNA. Five STRs have been identified in black swans (Cam1, Cam2, Cam3, Cam4 and Cam5). Using PCR, these five regions were amplified in all of the adults and cygnets from eight families of swans. The PCR products were then separated using agarose gel electrophoresis. Figure 6.50 shows the resulting gel. Each individual has two alleles for each STR, but sometimes only one band is observed (if the individual has two identical alleles).

Compare the profile of the mother of each family with the profile of each cygnet in her family and determine whether the female could have been the biological mother.

Results

Copy the table below and record the results in the second column.

FAMILY	BIOLOGICAL CYGNETS	PARASITIC CYGNETS
1		
2		
3		
4		
5		
6		
7		
8		

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1. Calculate the proportion of parasitic cygnets in each family and include the results in the third column.
2. Does there appear to be any difference in the proportion of parasitic cygnets between small and large families?

Analysis of results

1. Using your results, identify any evidence of brood parasitism in black swans.
2. Calculate the maximum proportion of parasitic cygnets in this sample.

Discussion

1. Explain whether your results support the belief that large black swan families are the result of brood parasitism.
2. Describe how you could determine whether a cygnet has been fathered by a male other than its social father.

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6.1 INVESTIGATION

Bacterial transformation

Background

DNA can mutate spontaneously or after an error is made in DNA replication. Biotechnologists have developed methods of controlling DNA mutation, intentionally mutating cell DNA to alter how the cell behaves. In addition, it is possible to transfer DNA from one organism into another. This is called genetic transformation, and it uses an engineered molecule of DNA to transfer a gene or genes from one organism to another so that the target organism is capable of producing the protein encoded by the transforming gene.

Aim

To perform a bacterial transformation using the green fluorescent protein plasmid pGreen

Time requirement

50 minutes

Materials

- *Escherichia coli* (*E. coli*) MM294 starter plate
- 10 µL pGreen plasmid
- 2 Luria broth (LB) agar plates
- 2 LB with ampicillin agar plates
- 10 mL LB, sterile
- 10 mL CaCl2, 50 mmol L^{-1}, sterile, ice cold
- 2 transformation tubes, sterile
- 8 plastic pipettes, 1 mL, sterile
- 3 inoculation loops, sterile, disposable
- 4 inoculation spreaders, sterile, disposable
- 2-20 µL variable micropipette
- Sterile tips for 2-20 µL micropipette
- Water bath
- Ice bath
- Fine-point marker pen

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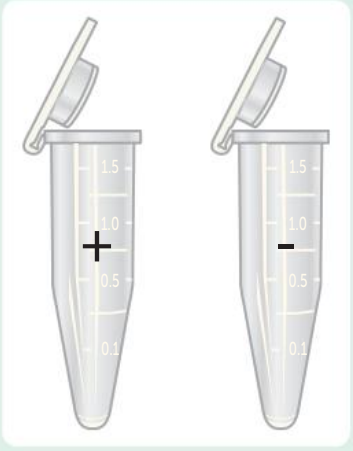
- Stopwatch
- Microtube rack
- Adhesive tape (to seal plates)
- Thermometer
- Incubator
- Ethanol
- PPE: lab coats, safety glasses, disposable glove

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Some bacteria may cause disease, so assume them to be pathogenic. (Note: *E. coli* MM294 is a harmless school-safe biological)	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end of activity. Decontaminate benches before and after activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Disinfectants or bleach may leave a corrosive residue.	After wiping the bench clean with bleach, ensure the residue is wiped off; ensure lab coat sleeves are rolled down and gloves are worn.

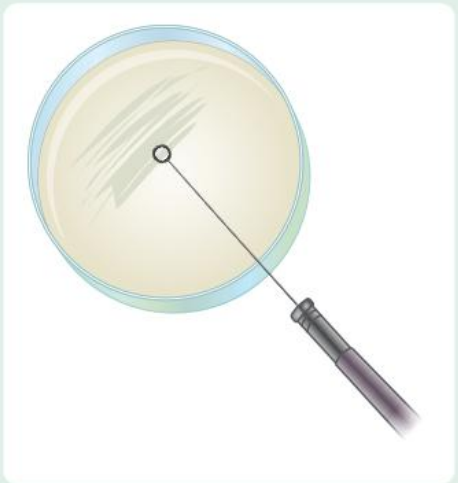
Procedure – Preparing the transformation solution

To use aseptic technique, wipe your bench down with ethanol (or bleach) and keep your work near the Bunsen burner to waft potential contaminants away from your materials.



1. Label one transformation tube '+ plasmid' and the other '– plasmid'. Keep the tubes cold by placing them upright in the ice bath. Tubes should be kept capped at all times except when in use.
2. Add 250 µL of ice-cold $CaCl_2$ solution to each transformation tube, using a sterile plastic pipette. Maintain the temperature by placing the tubes back into the ice bath.

Procedure – Suspending the bacteria



1. Transfer a single colony of *E. coli* from the starter plate to the ice-cold $CaCl_2$ solution in the '+ plasmid' transformation tube using a sterile inoculation loop. To dislodge the *E. coli* cells from the loop, spin the loop rapidly in the solution. Observe whether the cell mass has transferred successfully.
2. Immediately pump the liquid in the tube several times to suspend the cell mass in the $CaCl_2$ solution using a sterile 1-mL transfer pipette. Do not entrain air bubbles in the liquid or allow any liquid to splash up the sides of the tube. You should see the solution begin to become milky white as the cell mass is suspended. To check there are no lumps or particles in the tube, hold it up to the light; then return the tube to the ice.
3. Repeating the same steps as for the '+ plasmid' transformation tube, transfer a single colony of *E. coli* from the starter plate to the ice-cold $CaCl_2$ solution in

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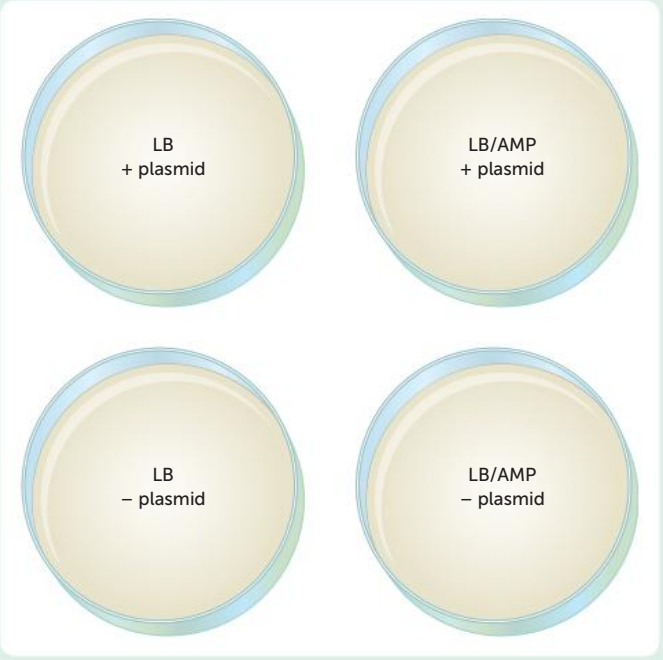
the '– plasmid' transformation tube using a sterile inoculation loop. To dislodge the *E. coli* cells from the loop, spin the loop rapidly in the solution. Observe whether the cell mass has transferred successfully.

4 Immediately pump the liquid in the tube several times to suspend the cell mass in the $CaCl_2$ solution using a sterile plastic pipette. Do not entrain air bubbles in the liquid or allow any liquid to splash up the sides of the tube. You should see the solution begin to become milky white as the cell mass is suspended. To check there are no lumps or particles in the tube, hold it up to the light; then return the tube to the ice.

Procedure – Adding the plasmid

1 The technician or your teacher will bring the plasmid to your work station. Transfer 10 µL of plasmid solution to the transformation tube labelled '+ plasmid' using a micropipette. Add the plasmid directly to the liquid in the tube without allowing it to touch the sides.
2 Immediately return the tube to the ice bath and mix the plasmid into the bacterial suspension by rapidly spinning a sterile inoculation loop with your fingers. Incubate the two tubes for 15 minutes on ice.
3 Label the four plates as follows:
The first LB plate: 'LB + plasmid'
The second LB plate: 'LB – plasmid'
The first LB/Amp plate: 'LB/Amp + plasmid'
The second LB/Amp plate: 'LB/Amp – plasmid'



Procedure – Heat shock

1 Extract the two tubes from the ice bath, transfer them to the warm water bath (42°C) and hold them there for 90 seconds with your gloved hands, keeping the tube caps from being fully submerged in the water. Gently agitate the tubes while they are warming up in the water. Immediately move the tubes back to the ice bath when the time is up.
2 Allow the tubes to rest in the ice bath for at least 1 minute before continuing.

Procedure – Recovery

1 Add 250 µL of Luria broth to each tube using a sterile plastic pipette. Mix the liquids at the base of each tube by gently grasping the top and tapping the base with your finger.
2 Allow the tubes to recover in the microtube rack at room temperature for 10 minutes.

Procedure – Plate inoculation

1 Transfer two drops of liquid from the '+ plasmid' tube to the 'LB + plasmid' plate using a sterile plastic pipette. Quickly spread the liquid evenly over the plate surface using a sterile spreader.
2 Transfer two drops of liquid from the '+ plasmid' tube to the 'LB/Amp + plasmid' plate using a sterile plastic pipette. Quickly spread the liquid evenly over the plate surface using a sterile spreader.
3 Transfer two drops of liquid from the '– plasmid tube' to the 'LB – plasmid plate' using a sterile plastic pipette. Quickly spread the liquid evenly over the plate surface using a sterile spreader.

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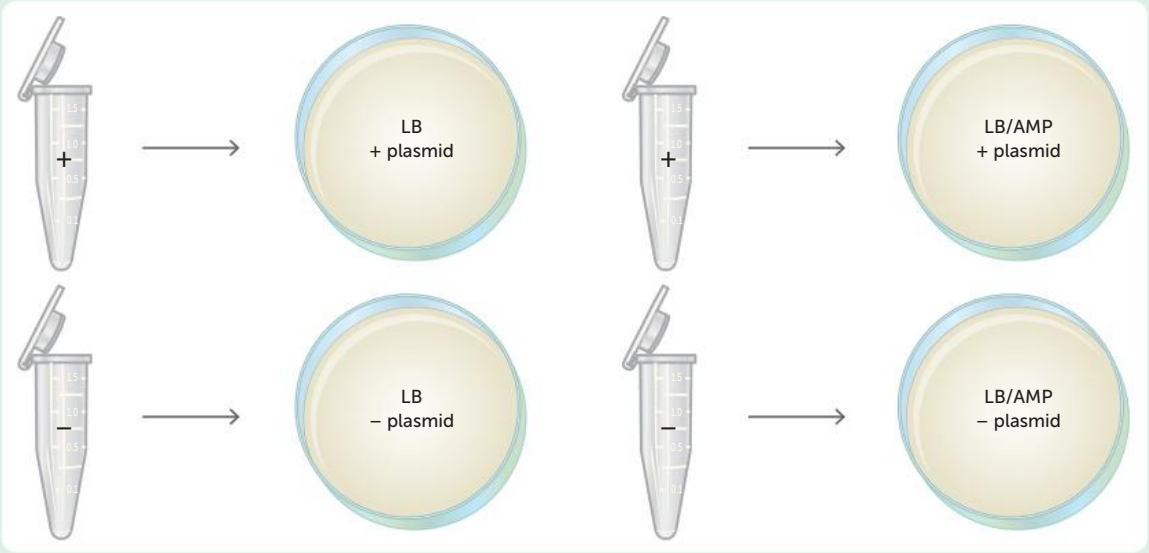
4 Transfer two drops of liquid from the '– plasmid' tube to the 'LB/Amp – plasmid' plate using a sterile plastic pipette. Quickly spread the liquid evenly over the plate surface using a sterile spreader.



5 Secure the lid of each petri dish to its base using tape. Leave the plates to rest on the bench for 5 minutes and then place them in a 33°C incubator for 24–36 hours. You can inspect the growth after this time. You should see either a bacterial lawn, single colonies, or no growth on the individual plates. Take the plates into a dark room to observe evidence of fluorescence in the transformed colonies. (Use of a UV light may enhance the fluorescence.)

6 To count the number of individual colonies, mark each colony with a marker as you count it. Any plates with cell growth too dense to count as individual colonies can be marked as a 'lawn'.

Results

1 Record the results of your experiment in a copy of Table 6.8 below.

TABLE 6.8 Bacterial colony results

PLATE	RESULT
- Plasmid on LB agar	
+ Plasmid on LB agar	
- Plasmid on LB/Amp agar	
+ Plasmid on LB/Amp agar	

2 What growth and phenotypes can you observe?

3 Describe what you see on your plates when you look at your plates under UV light?

Discussion

1 Why is the plasmid–bacteria solution placed on ice for 5 minutes?

2 Which plate forms the control in this experiment? Explain.

3 Explain the function of the Luria broth. What is the purpose of incubating the cells at room temperature?

4 Explain how the DNA plasmid is put into bacteria. What is the advantage of being able to do this? Consider what the plasmid DNA allows the bacteria to do.

5 Explain how we are able to recognise that the plasmid DNA is in the bacteria.

6 Explain what a plasmid is.

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Conclusion

Summarise your findings and discuss your results.

Taking it further

Investigate how genetic engineering and bacterial transformation enable the advancement of medical treatments; for example, in insulin production.

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6.2 INVESTIGATION

The effect of restriction digestion enzymes on Lambda DNA

Background

Restriction digestion is the process of cutting DNA molecules into smaller pieces with special enzymes called restriction endonucleases (or restriction enzymes). These special enzymes recognise specific sequences in the DNA molecule (e.g. EcoRI GAATTC) wherever that sequence occurs.

Aims

To use restriction enzymes to cut DNA into fragments
To analyse restriction digestion using gel electrophoresis apparatus

Time requirement

55 minutes

Restriction digestion materials

- 8 µL Lambda DNA (1 µg)/µL
- 20 µL restriction digestion buffer
- 1 µL EcoRI enzyme
- 1 µL HindIII enzyme
- 1 µL BamHI enzyme
- 200 µL sterile nuclease-free water
- 4 sterile 0.5 mL (500 µL) microtubes
- 5–50 µL variable pipette
- 0.5–10 µL variable micropipette
- Microtube rack
- Sterile pipette tips
- Water bath
- Micro-centrifuge (optional)
- PPE: lab coats, safety glasses, disposable gloves

Electrophoresis materials

- 25 µL TBE buffer
- 0.8% agarose gel with 2 µL of Midori green safe stain (for pre-staining technique)
- 50 µL loading dye
- 2–20-µL variable micropipette
- Electrophoresis chamber (blue-gel)
- Power supply 100 V (if using an alternative to blue-gel)
- Blue-light transilluminator (optional)

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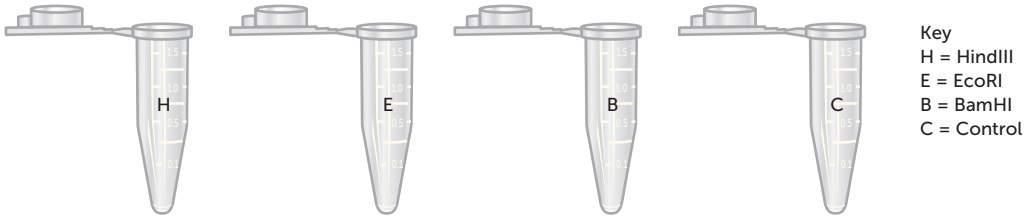
Please note: the above measurements are based on using a blue-gel electrophoresis apparatus. If an alternative electrophoresis chamber is being used, increase the TBE quantities based on chamber size.

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
TBE buffer can cause skin irritation.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves may pose allergy risk.	Use a type of glove that has no allergy risk and is suitable for the chemicals being used.

Procedure – Restriction digestion

1 Collect four 0.5 mL microtubes, and label them as follows:



2 Using a micropipette, add 42 µL of nuclease-free water to each of the microtubes.
3 Add 2 µL of Lambda DNA to each of the microtubes.
4 Using a fresh micropipette tip, add 5 µL of restriction digestion buffer to microtubes 'E', 'B', 'H' and 'C'.
5 Using a fresh micropipette tip for each sample, add 1 µL of the EcoRI enzyme to 'E', 1 µL of the BamHI enzyme to 'B', 1 µL of the HindIII enzyme to 'H' and 1 µL of nuclease-free water to 'C'.
6 Mix the samples thoroughly by pipetting up and down a few times using the larger micropipette with fresh tips for each sample. Continue until the samples have an even consistency. To collect the liquid at the base of the tubes, spin with a microcentrifuge.
7 Place the microtubes in a 37°C water bath for 10 minutes.

Procedure – Analysing your digestion using gel electrophoresis

1 Collect the four tubes from the water bath and add 10 µL of loading dye to each sample.
2 Mix the samples thoroughly by pipetting up and down a few times using the larger micropipette with fresh tips for each sample until the solutions look consistent throughout. To collect the liquid at the base of the tubes, spin with a micro-centrifuge. Your samples are now ready to be loaded into the gel.
3 Place the prepared 0.8% agarose gel into the gel electrophoresis chamber, ensuring that the wells are at the top or negative electrode section of the chamber.
4 Pour TBE buffer into your gel electrophoresis chamber, ensuring the surface of the gel is completely covered.
5 Using a fresh pipette tip for each sample, load 10 µL of each of your restriction digest samples into the wells located near the negative electrode and note the specific lanes into which the different samples were loaded.
6 Carefully place the lid on the gel chamber, press the 'on' button and let the gel run for 30 minutes. (Turn on the built-in blue light to visualise the DNA band separation if using a blue-gel electrophoresis chamber.)

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Note: if using a gel electrophoresis chamber that requires an external power supply, carefully plug the positive and negative electrodes into the gel box without dislodging the gel. The negative end should be connected to the end closest to the DNA samples. Plug the power source in (set at 100 V), turn it on and let the gel run.

7 After 30 minutes has passed, turn the power supply off and visualise your results either by turning on the blue light or transferring the gel to a blue-light transilluminator.

(Note: if you are using the post-strain method, the DNA will not be visible until the gel has been soaked in methylene blue or an equivalent dye for up to 24 hours.)

Results

1 How many cuts did each restriction enzyme make?
2 Measure the distance travelled by the DNA fragments in millimetres and fill in a copy of the table below.
3 Graph your results for HindIII digest to determine the sizes of the EcoRI digest and or BamHI digest. [Try graphing the Log (base pairs) vs distance.]
4 Do those fragments add up to the size of Lambda DNA? If not, provide a possible explanation(s) as to why not.

Analysis of restriction digests of DNA

HindIII		EcoRI			BamHI		
DISTANCE (mm)	SIZE (BP)	DISTANCE (mm)	CALCULATED NO. OF BP	SIZE (BP)	DISTANCE (mm)	CALCULATED NO. OF BP	SIZE (BP)
	23 130			21 226			16 841
	9 416						
	6 557						
	4 361						
	2 322						
	2 027						

Discussion

1 Why was 1 µL of nuclease-free water added to the microtube labelled 'C'?
2 Why do we incubate the restriction digests at 37°C?
3 What is the purpose of the dye?
4 What would occur if the gel electrophoresis chamber was filled with distilled water instead of TBE buffer?
5 Explain why DNA samples must be loaded at the negative end of a gel electrophoresis chamber.
6 What would occur if the electrodes in the electrophoresis chamber were reversed?

Conclusion

Summarise your findings and discuss your results.

Taking it further

Investigate real-world examples of where restriction enzymes are used and how they assist in medical disease diagnosis.

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CHAPTER 6 SUMMARY

Chapter 6 Activity sheet

- Biotechnology provides ways to improve our lives and the health of our planet by extracting, analysing and manipulating DNA to make useful products.
- DNA biotechnology uses tools such as restriction enzymes, plasmids, vectors and microarrays.
- DNA biotechnology techniques include DNA sequencing, PCR, gel electrophoresis and recombinant DNA.
- Restriction enzymes are enzymes isolated from bacteria that cut DNA at specific sites known as restriction sites. These sites are four to eight nucleotides long. Cutting can result in the formation of either sticky ends or blunt ends.
- DNA ligase is an enzyme used to join two DNA molecules with complementary sticky ends or with blunt ends.
- DNA polymerase uses complementary base pairing to synthesise new fragments of DNA.
- Primers use complementary base pairing to mark a target strand of DNA, showing DNA polymerase where to begin synthesis.
- PCR is a process through which a specific DNA sequence can be amplified for analysis.
- Using gel electrophoresis, it is possible to separate DNA fragments according to size and to visualise them by using a DNA-binding dye that fluoresces under UV light.
- Gene probing uses a single-stranded DNA molecule to identify, isolate or find the position of a gene on a chromosome. Multiple probes form a microarray and help scientists measure gene expression.
- DNA sequencing allows determination of the exact nucleotide sequence of DNA fragments. Sequencing can help to identify genetic mutations that cause disease, and also enable gene mapping.
- DNA profiling is a technique that can be used to create individual genetic profiles in order to differentiate between the DNA of individuals of the same species.
- Genes can be transferred from one organism to another using different vectors, including plasmids and viruses, using recombinant DNA techniques.
- Gene cloning is a process through which a large number of copies of a gene of interest can be made in bacteria by incorporating the gene in a plasmid.
- Transgenic organisms (genetically modified organisms) have genes from foreign DNA inserted into their genome.

CHAPTER 6 GLOSSARY

Agarose gel The medium commonly used for electrophoresis of proteins and nucleic acids. It allows these molecules and an electric current to flow through it, but also acts as a sieve, sorting out the different-sized fragments; shorter DNA fragments migrate through the gel more quickly than longer ones

Annealing In PCR, annealing is the process of joining separate strands of DNA together as a result of hydrogen bond pairing; it occurs when the temperature is lowered

Band on the gel A well-defined line in a lane on a gel; it contains millions of pieces of DNA of the same size

Biotechnology The use of living organisms and biological systems and processes for human benefit

Blunt end The end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

Denature In PCR, denaturing is changing the molecular structure of a protein or DNA by applying a high temperature; in DNA, the hydrogen bonds break and the two strands separate

DNA ligase An enzyme used to catalyse the formation of a bond between two pieces of DNA

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DNA polymerase A member of a class of enzymes found in all living things, which synthesises new strands of DNA based on a template strand and according to complementary base-pair rules; DNA polymerases are important tools in biotechnology because they are capable of making exact copies of fragments of DNA, enabling efficient and accurate amplification of DNA templates

DNA profiling A process that is able to identify natural variations that exist within individual genomes, by using STRs, PCR and gel electrophoresis

DNA sequencing The process of establishing the nucleotide sequence of a piece of DNA

Ethidium bromide a chemical that binds to double-stranded DNA and fluoresces pink when exposed to ultraviolet light; used to locate DNA in an agarose gel following electrophoresis

Gel electrophoresis A technique that separates large molecules (either fragments of DNA or proteins) according to their size and charge, for visualisation and identification purposes

Gene cloning The process of using plasmids and bacteria to make numerous identical copies of a gene

Gene expression The translating of a gene into a protein by an organism; the phenotype is directly affected by gene expression

Gene probe A specific short length of single-stranded DNA that can bind to a particular gene of interest

Genetic engineering Manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

Genetic mapping Identifying and recording the relative positions of genes on a chromosome using **genetic markers**, **linkage mapping** and **physical mapping**

Genetic marker A nucleotide sequence that is associated with a specific trait; genetic markers may include short DNA sequences, such as STRs, or they may be whole genes

Genetically modified organism (GMO) See **transgenic organism**

Genome All of the genetic material contained in an organism or a cell; it includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

Ladder A standard collection of DNA fragments of known lengths (**molecular size markers**) used in gel electrophoresis

Lane A corridor through which DNA passes after it leaves a well

Linkage mapping Using frequencies of genes that cross over together to determine the relative positions of genes on a chromosome in **genetic mapping**

Micropipette A tool that dispenses small amounts of samples into PCR tubes or into gel wells; the volume is adjustable

Molecular size marker A piece of DNA of known length; a set of molecular size markers are run alongside the samples in a gel to estimate the size of the DNA fragments in the sample (see **ladder**)

Next-generation sequencing (NGS) An automatic process that finds the order of nucleotides in a strand of DNA. The four nucleotides are labelled with four differently coloured fluorescent dyes. As electrophoresis proceeds, a laser scans across the bottom of the gel, detecting the different dyes and thus the base sequence. A computer can then automatically analyse the information from the gel to read the base sequence.

Physical mapping A precise description of a gene's position on a chromosome; the gene's position is measured and located by the use of base pairs

Plasmid A small circular piece of DNA, found in bacteria that is able to replicate independently of the cell's chromosomes; engineered plasmids can carry antibiotic-resistance markers

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Polymerase chain reaction (PCR) A cyclic method used to rapidly amplify (replicate) relatively small amounts of DNA into extremely large amounts, for further laboratory uses such as gel electrophoresis and DNA profiling

Primer A short fragment of single-stranded nucleic acid (DNA or RNA); primers can be made in a laboratory, are about 20 nucleotides long and are usually labelled with an enzyme, or radioactive or fluorescent dye tag; a primer is attracted to a target DNA strand by complementary base pairing and marks where elongation/synthesis should start

Recognition site A specific sequence of DNA at which a restriction enzyme will cut

Recombinant DNA technology Tools and techniques used to transfer a gene from a cell of a member of one species to the genome of a different species

Recombinant plasmid A plasmid with foreign DNA inserted into it

Restriction endonuclease (restriction enzyme) An enzyme that cuts DNA at a specific restriction site

Restriction enzyme See **restriction endonuclease**

Restriction fragment A short fragment of DNA generated when a restriction enzyme cuts a longer DNA sequence

Restriction site A specific nucleotide sequence, usually 4–8 base pairs long, that is recognised as a cleaving site for a restriction enzyme

Short tandem repeat (STR) A short non-coding region of DNA that is repeated many times in the genome of an organism; it is highly variable between individuals and can be used in DNA profiling; an STR has a repeat sequence of two to five bases

Sticky end The end of a DNA fragment that is created when a restriction enzyme cuts DNA at different positions on each of the two strands

Thermal cycler A machine used in PCR that provides tight control over both the reaction temperature and the duration of each temperature step, ensuring efficient amplification

Transformation The process by which DNA is taken from one organism and inserted into another organism, usually of another species, to obtain a desired characteristic

Transgenic organism An organism that has been modified by incorporating into its genome a piece of foreign DNA; also called a **genetically modified organism (GMO)**

Variable nucleotide tandem repeats (VNTRs) Short non-coding regions of DNA that are repeated many times in the genome of an organism; they are highly variable between individuals and can be used in DNA profiling; VNTRs have a repeat sequence of more than five bases

Vector In medicine, a vector is an agent that transmits pathogens from one host to another; in genetics, it refers to a vehicle used to transfer DNA sequences from one organism to another

Well An indentation in a gel used in a gel electrophoresis apparatus. It is made by inserting a comb into the gel as it sets and is placed at the negatively charged end of the apparatus. DNA samples and a standard are pipetted into a row of wells

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CHAPTER 6 REVIEW QUESTIONS

Remembering

1 Match each item in the first column with a description in the second column. Each item can only be used once.

DNA ligase	Small circular self-replicating DNA molecule
Vector	Sorts DNA molecules based on size and charge
Primer	Joins two single-stranded sections of DNA together
Sticky ends	Specific site at which restriction enzymes cut DNA
Plasmid	Vehicle to introduce DNA into a host cell
Restriction site	An enzyme that catalyses the synthesis of DNA
Gel electrophoresis	Result from cleavage by a restriction enzyme at different positions on the two strands of DNA
DNA polymerase	Synthetic short, single-stranded DNA molecule

2 Recall the two most common virus vectors.

3 State why the temperature is lowered to 50–60°C during the annealing phase of PCR.

Understanding

4 Predict whether the following cuts made by restriction enzymes will produce sticky or blunt ends. The arrows show where the cuts occur in the double-stranded DNA.

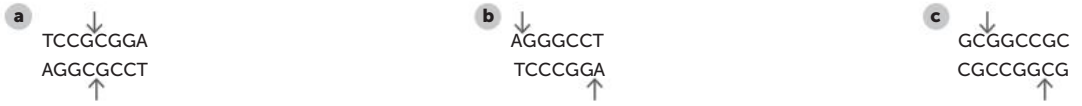

FIGURE 6.45 Restriction enzymes cutting DNA

5 Summarise why radioactive or fluorescent tags are added to gene probes.

6 Outline the major disadvantage of using viruses as vectors to transfer genes from one organism to another.

Applying

7 Predict the minimum band-sharing percentage in the DNA profiles of a mother and her baby.

8 Look carefully at the gel in Figure 6.47. Based on the figure, match the size of fragments in lanes 1, 2, 3 and 4 to the sets of measurements presented below.

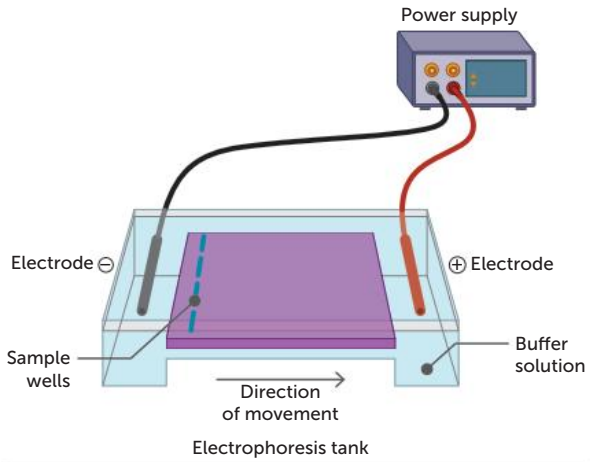

FIGURE 6.46 DNA electrophoresis kit

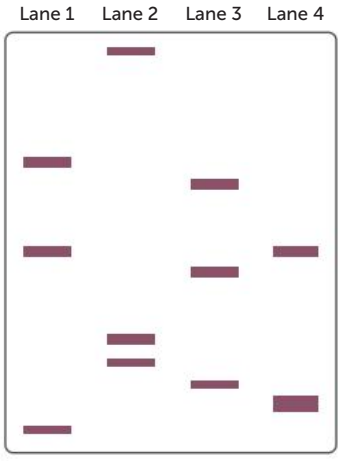

FIGURE 6.47 Electrophoresis gel

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a 200, 250 and 900 bp
b 150, 400 and 600 bp
c 50, 450 and 650 bp
d 100, 100 and 450 bp

9 Predict whether digestion of the human genome with AluI or EcoRI would result in the larger number of fragments.

10 The section of DNA in Figure 6.48 shows a sequence of 120 bases in one strand of DNA. Refer to Table 6.2 on page 171 for restriction sites.

```
ATATGTGT GGATCCGT CTTAGGTT ATCGAATT CTAGAGCT
ATGGCCTA TTAGCTTC CTGGATCC AACCTGTA TAGAGCTA
CTCGTCAG CTATTGCT ACGGGATC CTAGCTGA TTGGATTC
```

FIGURE 6.48 DNA sequence

a How many BamHI and AluI restriction sites are there in the sequence?
b If the sequence is cut by BamHI, how many kinds of fragments of DNA would be produced?
c If the sequence was cut by AluI, how many kinds of fragments of DNA would be produced?
d If the sequence was cut by both BamHI and AluI, how many kinds of fragments would be produced?
e If the piece of DNA was circular and not linear, how many cuts would have been made by BamHI to get the number of fragments stated in part **b**?

11 Looking at the profiles (Figure 6.50, page 212) of the black swan family of eight (family 3, Figure 6.49), determine whether the male is the biological father of all cygnets.

Dreamstime/Hbfiala

FIGURE 6.49 A black swan family

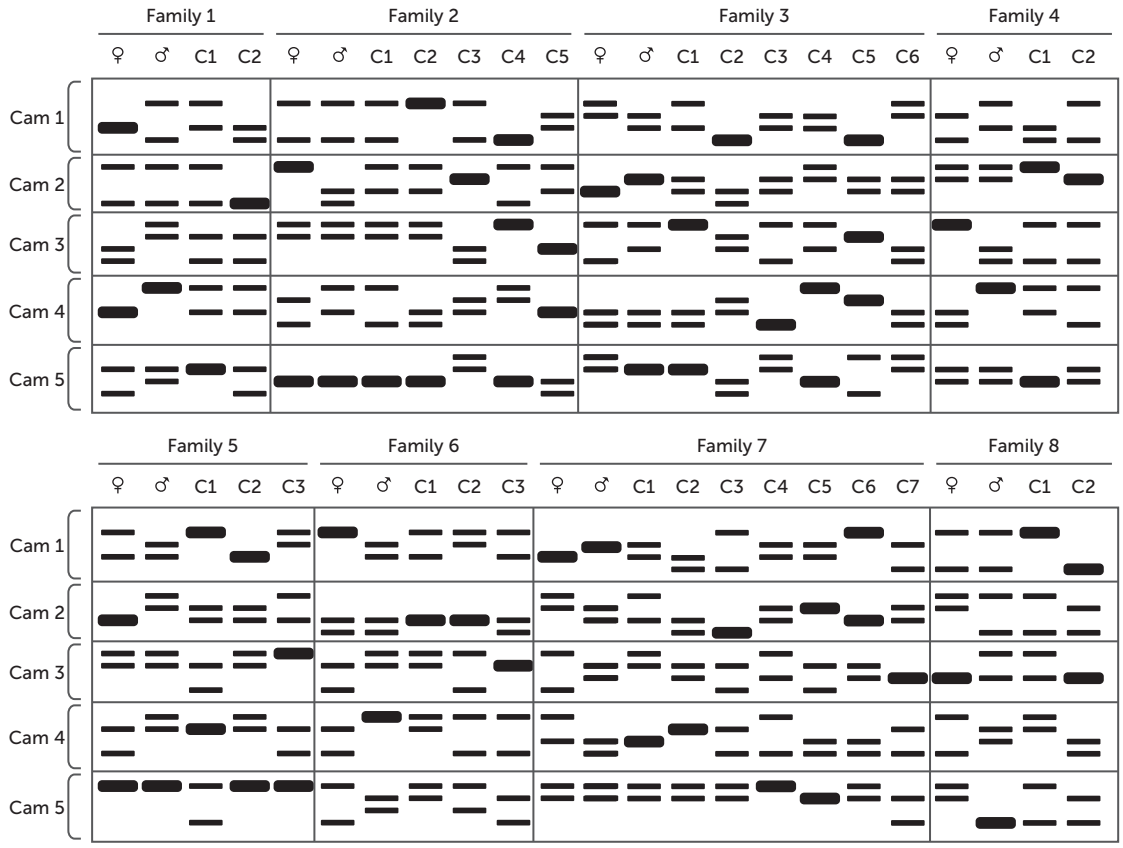

FIGURE 6.50 DNA profile for eight black swan families, which each include a social mother (♀), a social father (♂) and cygnets (C)

Analysing

12 Identify some of the instances when only a small sample of DNA may be available.

Creating

13 Based on the knowledge you have acquired while studying this chapter, design a way to test for a mutation resulting in the deletion of a region of 100 nucleotides that does not contain any restriction sites.

14 Explain how you would use DNA profiling to design a breeding program that minimises inbreeding of an endangered species.

Reflecting

15 Complete the following sentences

- **a** Before I started studying this topic, I thought biotechnology involved …
- **b** After studying this topic, I think biotechnology involves …

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PRACTICE EXAM QUESTIONS

1 Which of the following statements about the movement of DNA fragments through an agarose gel is correct?

- **A** Larger fragments move faster than smaller fragments because it is easier for them to move through the gel.
- **B** Larger fragments move faster than smaller fragments because they have a higher negative charge.
- **C** Smaller fragments move faster than larger fragments because it is easier for them to move through the gel.
- **D** Smaller fragments move faster than larger fragments because they have a lower negative charge.

[Q8 2019 SCSA]

2 In genetic engineering, plant viruses are sometimes used to introduce a foreign gene into a plant cell. This is because viruses are:

- **A** non-living and therefore easy to store in the laboratory
- **B** non-living and therefore there are no ethical issues with using them in this way
- **C** able to invade the cell and produce a large number of viral particles very quickly
- **D** able to invade the cell and merge their genetic material with that of the cell.

[Q26 2018 SCSA]

3 In gene cloning, the main purpose of plasmids is to

- **A** identify the gene for cloning
- **B** extract the desired gene from a donor organism
- **C** produce many copies of the desired gene
- **D** introduce the desired gene into a recipient organism.

[Q10 2015 SCSA]

4 Which row in the following table correctly matches an enzyme with its function?

[Q21 2015 SCSA]

	ENZYME	FUNCTION
A	DNA polymerase	Cuts a DNA molecule at a specific sequence
B	RNA polymerase	Degrades RNA molecules
C	Ligase	Joins two DNA molecules together
D	Restriction enzyme	Synthesises a new strand of DNA

5 A breeder kept only albino guinea pigs. The breeder put one female and two male guinea pigs in the same enclosure. The female had a litter of offspring. The breeder wanted to know which of the male guinea pigs was the father of the litter. Explain how biotechnology can be used to determine the father of the litter. (4 marks)

[Q33e 2018 SCSA]

6 List the main steps involved in producing a DNA profile for an organism. (4 marks)

7 A number of people who had visited a particular dental practice were later found to be infected with a hepatitis virus. Health authorities suspected that these people had contracted the virus through the dental practice. Explain how DNA profiling could be used to determine whether this was the case. (4 marks)

[Q35c 2016SCSA]

8 State the role that the following factors play in gene cloning. (4 marks)

- **a** restriction enzymes
- **b** ligase
- **c** plasmid
- **d** vector

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9 Chymosin is an enzyme produced by nursing calves to assist with the digestion of milk. Humans also use chymosin to make cheese. Traditionally, chymosin for cheesemaking was obtained from the stomach of calves that had been killed for their meat. It is now obtained from genetically modified microorganisms. Describe how recombinant DNA technology can be used to genetically modify bacteria to produce chymosin. (6 marks)

10 Artificial selection and transgenesis (the production of transgenic organisms) are two methods that humans use to change the features of plants or animals. Describe how artificial selection and transgenesis can each be used to change the features of plants or animals. (5 marks)

[Q38 2015 SCSA]

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7 BIOTECHNOLOGY IN AGRICULTURE AND ENVIRONMENTAL CONSERVATION

CHAPTER 7 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Do you know any plant or animal species that are endangered? Can biotechnology help save them?
2 How is biotechnology applied to agriculture? Are the modifications proving to be beneficial or harmful?
3 How do you benefit from environmental conservation?

SCIENCE UNDERSTANDING

» recombinant DNA technology and DNA identification technologies are applied in agriculture and environmental conservation

SCIENCE AS A HUMAN ENDEAVOUR

» transgenic organisms have been engineered for desirable traits, including resistance, faster growth rate, greater product quality and yield, and tolerance to adverse environmental conditions
» using transgenic organisms may have adverse effects on genetic diversity and the environment, including
  – the effects on non-target organisms
  – more rapid evolution of pesticide-resistant species
  – the possibility of gene flow from crop species to weed species resulting in the emergence of 'super weeds'
» biotechnology can be used in environmental conservation for
  – monitoring endangered species
  – assessing gene pools for breeding programs
  – quarantine
» conservation planning to maintain viable gene pools includes consideration of
  – biogeography
  – reproductive behaviour
  – population dynamics

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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7.1 DNA IDENTIFICATION TECHNOLOGIES IN AGRICULTURE

Agriculture is the science of growing crops and livestock, and cultivating the soil and micro-organisms in which they grow.

In the past several decades, science has allowed us to decipher much of the genetic code of the crops and animals that are farmed. Instead of traditionally crossbreeding corn plants to determine which traits cause them to, for example, grow taller or require less water, we can find the desirable traits in the corn plant's genetic map. Then we can use biotechnology to isolate the relevant gene and transfer it into another plant, thus producing a new variety of plant that possesses the beneficial characteristics.

Identification technologies can be used to accurately trace the genetics of desirable traits and to pass those traits to other plants within a generation. These techniques are enabling scientists to increase the availability and quality of food for our growing human population. To help increase the availability and quality of food, scientists call on identification technologies, including the tools and techniques used in building DNA profiles (fingerprints), such as analysing single tandem repeats (STRs). In addition, they use restriction enzymes, polymerase chain reaction (PCR), gel electrophoresis, DNA sequencing, gene markers and genome mapping.

Using **marker-assisted breeding**, plant scientists can examine the DNA of seeds to find the ones that will produce the best plants. First, genetic markers are identified in a plant's DNA that are linked to important traits such as disease resistance, drought tolerance, **yield**, taste or nutrition. The markers are then used to screen all of the plants available for breeding and accurately select and breed only the seeds that will produce plants with the desirable traits.

Application to wheat breeding

Wheat is an important staple, not only in human diets, but also in livestock feeds. It is the world's most widely cultivated crop. Leaf rust (*Puccinia triticina*) is a fungal leaf disease specific to wheat that can pose a significant threat to the yield and quality of WA wheat crops, in some seasons causing up to 30% yield loss in susceptible varieties. Yellow (stripe) rust (*Puccinia striiformis*) is another globally important disease in wheat. Researchers at CSIRO have used PCR and gel electrophoresis to reveal molecular markers for characterising loci that confer resistance in the adult plant to leaf rust and yellow (stripe) rust. They have also established a biotechnology laboratory that is charged with acquiring, validating and applying markers for certain traits that are important to wheat breeders. Use of PCR-based markers, coupled with rapid DNA extraction procedures, has enabled the application of markers to a wide range of material. Genetic engineering procedures have also been used to establish procedures for experimenting with genes that confer resistance to various biotic and abiotic stresses in wheat.

Alamy Stock Photo/robertharding

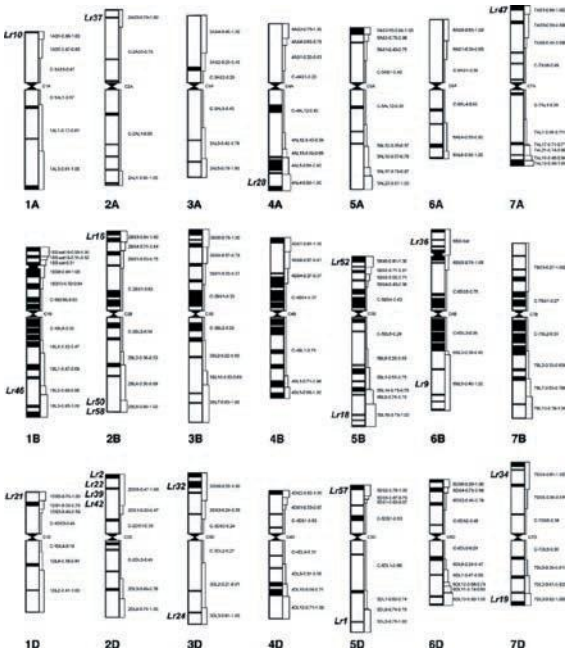
Shutterstock.com/aleori

FIGURE 7.1 a A healthy wheat crop; **b** wheat leaf displaying signs of wheat rust disease

To meet the future demands of a projected world population of 9.6 billion by 2050, wheat productivity needs to increase by 1.6% each year. To preserve biodiversity, water, and nutrient resources, the majority of this increase has to be achieved via crop and trait improvement on land currently being cultivated, rather than by committing new land to cultivation.

The wheat genome was sequenced and published in 2018, and has enabled scientists to undertake more accurate mapping of desirable genes, such as disease-resistance genes.

With the reference genome sequence completed, breeders have new tools for addressing these challenges at their disposal. They can rapidly identify genes and regulatory elements underlying complex agronomic traits such as yield, grain quality, resistance to fungal diseases, and tolerance to abiotic stress – and produce hardier wheat varieties. CSIRO has used PCR and gel electrophoresis to reveal PCR markers that are now assisting farmers in identifying genes that improve rust resistance.



Reproduced from Gill et al. (2008) with permission from CSIRO Publishing.

FIGURE 7.2 Approximate locations of mapped leaf rust–resistance genes on a map of wheat chromosomes

Application to the pork industry

The Government of Western Australia issued the Western Australian Pork Industry Strategic Plan 2012–20 to increase consumption of fresh pork, increase productivity, reduce costs across the supply chain, and develop and grow export markets for pork. DNA identification technologies play an important role in the genetic management systems of this plan. The larger breeding companies use computer programs like PIGBLUP to monitor the progress of their breeding program. BLUP is an acronym for Best Linear Unbiased Prediction, and it can accelerate the rate of genetic progress (especially for traits of low heritability, such as litter size) by giving a more accurate measure of each animal's breeding value.

PBMARKER takes into account information on molecular genetic markers in genetic evaluation. Using molecular genetic information aids selection in economically important traits. These identification technologies allow pig farmers to select the pigs with the most desirable traits for breeding. Desirable traits include increased litter size and growth rate, and improved carcase quality (e.g. taste and tenderness). A side effect of selecting genetically superior animals is the increase in genetic relatedness and decreasing genetic diversity within the population. More information on breeding programs is available from the Animal Genetics and Breeding Unit based at the University of New England in New South Wales.

Shutterstock.com/Thuwanan Krueabudda

FIGURE 7.3 Breeding superior pigs to increase productivity

Animal Genetics and Breeding Unit
For further information about animal genetics and breeding, visit the Animal Genetics and Breeding Unit website developed by the University of New England in New South Wales.

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Key concept

Biotechnology is used in agriculture to improve yield, quality and productivity of crops and animals. However, this can result in reduced genetic diversity in the population.

Question set 7.1

REMEMBERING

1 Define agriculture.
2 List three types of DNA identification technologies used in agriculture.

UNDERSTANDING

3 Describe the use of DNA identification technologies in breeding:
   a wheat
   b pigs.
4 In your answer to Question 3, state the desired trait(s) being pursued and the reason(s) why they are desired by farmers.

7.2 RECOMBINANT DNA TECHNOLOGY IN AGRICULTURE

Millions of people around the world are malnourished. Scientists have been using molecular tools and techniques to modify food crops, resulting in increases in their nutritional value and crop yields. Biotechnology has also been applied to reducing the impact of pests on crops, thus increasing the amount of food available in developing countries. The process most commonly used is **transformation**: taking a gene from one species and inserting it into another to obtain a desired characteristic. The technology used for this process is termed **recombinant DNA technology**. **Transgenic organisms**, also known as **genetically modified organisms (GMOs)**, have been engineered for desirable traits, including disease resistance, faster growth rate, greater product quality and yield, and tolerance to adverse environmental conditions. **Bioengineering** is the combination of biology and engineering tools to create a usable product like a transgenic organism.

The transfer of a desirable gene can be via a gene gun or a viral vector. Another interesting method uses the soil bacterium *Agrobacterium tumefaciens*. This bacterium has evolved the ability to penetrate cell walls with its plasmid and insert specific genes into the genome of the host plant cells. The Ti plasmid in *A. tumefaciens* causes an infectious disease in plants known as crown gall disease (see chapters 12–14). Scientists have learned how to control and make use of this phenomenon in order to make GMOs that are desirable to humans. A desired gene is cut from a foreign source, inserted into the Ti plasmid to make a recombinant plasmid, and returned to *A. tumefaciens* for cloning. The bacterial vector can then be cultured with plant cells that are susceptible to penetration by the Ti plasmid. Once the plasmid has penetrated a host cell, the genes are be inserted, transforming the host plant into a genetically modified (GM) crop. The petri dish GMOs can then be cross-bred with breeding stock many times.

Transgenic organisms engineered for resistance

Herbicide-resistant crops

A major problem for farmers is the large number of weeds that grow throughout their crops. **Herbicides** are substances used to control weeds, ideally leaving a crop unharmed. They can be classified as selective or non-selective, depending on the types of plants they affect. Spraying herbicides on crops to kill the weeds usually damages the crop as well. Herbicide-tolerant crops have

been developed, which are proving to be an effective solution for farmers. These include canola, soybean and sugar beet. There are a few different GM soybeans grown in Australia. The popular glyphosate-resistant Roundup Ready® soybeans have a gene inserted into them from a bacterium. **Roundup Ready crops** are tolerant to the herbicide called Roundup Ready, which contains the active ingredient glyphosate. Glyphosate inhibits a biochemical pathway in plants, preventing them from producing essential amino acids and causing them to die.

Roundup is a brand name for the glyphosate herbicide. While Roundup is a great weed killer, its broad-spectrum effects make it a crop killer, too. A company named Monsanto was the first to make a GM soybean resistant to glyphosate. In this case, the gene providing resistance to glyphosate was taken from *Agrobacterium*.

Herbicide-resistant crops may be more productive and may be more environmentally sustainable, but their use is questioned by many. It is too soon to be sure of the long-term effects on the environment and on other organisms.

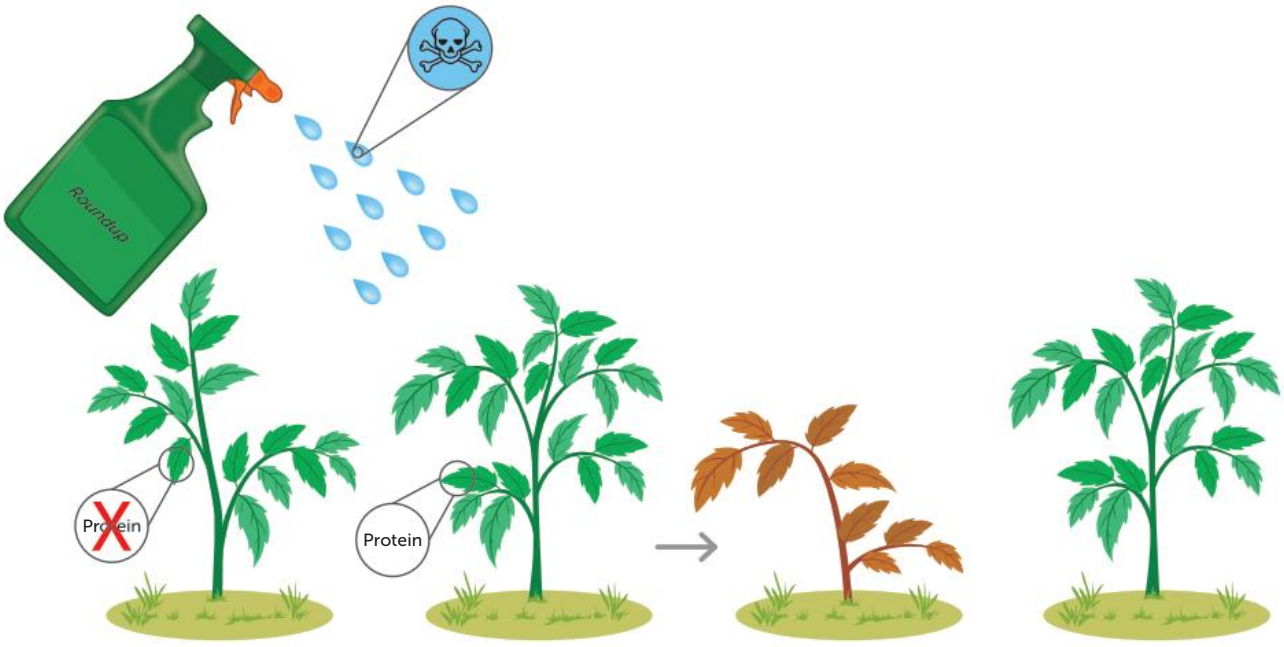

FIGURE 7.4 Roundup Ready crops are resistant to glyphosate. Regular crops and weeds are inhibited from making essential proteins.

Disease-resistant crops

Stem rust is a disease of wheat in eastern Australia. It is treated by spraying plants with fungicides. However, the pathogens that cause stem rust can develop resistance to fungicides, and new strains of the stem rust frequently appear. CSIRO has developed a method of isolating the rust-resistant gene *L6* from a rust-resistant flax plant and then introducing it into a rust-susceptible wheat plant. This genetically engineered plant grows with resistance to rust. Other similar examples include ringspot virus-resistant papayas and yellow mosaic virus-resistant zucchini.

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FIGURE 7.5 Bt cotton is resistant to cotton bollworm.

Cotton plants are susceptible to *Helicoverpa armigera*, more commonly known as the cotton bollworm. The larvae of this moth munch on precious crops in Asia, Europe, Africa and Australia,

causing damage estimated at greater than $2 billion each year. Normally, regular spraying of insecticide has been used to eradicate this pest. However, it has been discovered that the soil bacterium *Bacillus thuringiensis* (Bt bacteria) produces a range of proteins that are toxic to some insects. Genes that make proteins toxic to the bollworm are taken from this bacterium and inserted into cotton plants, and when expressed, the GM cotton produces the same toxin. The use of genetically engineered cotton reduces the use of insecticides, cutting farming costs. Unfortunately, resistant strains have evolved in various insects.

In providing farmers of GM cotton with alternative weed and pest management options, GM cotton could help reduce the environmental impacts that previous management of non-GM cotton crops has caused. Since 2011, WA farmers have been authorised to grow GM cotton commercially, and currently more than 99% of cotton grown in Australia is GM cotton.

Faster growth rate

The first GM animal in the world to be grown for human consumption was a transgenic Atlantic salmon known as AquAdvantage salmon®. It is capable of growing at double the rate of conventional Atlantic salmon. A more effective hormone-regulating gene normally found in the Pacific Chinook salmon replaced the normal hormone-regulating gene in Atlantic salmon. Both genes regulate growth. In addition, a promoter gene normally found in ocean pout was inserted into the Atlantic salmon. The purpose of the modifications was to increase the speed at which the fish grows, without affecting its ultimate size or other qualities (Figure 7.6). GM fish grow to market size in 16–18 months, rather than 3 years. The first US and Canadian harvests of the GM salmon are expected in 2020.

After an approval process that took nearly 20 years, the US Food and Drug Administration (FDA) said the GM salmon posed no health risks and they could find no reason not to approve it. However, the Australian food safety regulator, Food Safety Australia New Zealand (FSANZ), issued a statement to Australian consumers saying the GM salmon had not been approved for sale in Australia.

AAP Photo/AP Photo/Aqua Bounty Farms

FIGURE 7.6 GM and non-GM Atlantic salmon

Greater product quality and yield

Increase in quality (nutrition) in rice

The World Health Organization estimates that 124 million people suffer from **vitamin A** deficiency, and that one to two million die each year from this deficiency. The deficiency results in between 250 000 and 500 000 irreversible cases of blindness annually, mainly in children, half of whom die within a year of becoming blind. Most of these people live in urban slums, where poverty restricts their diet to a daily ration of rice. Vitamin A is a fat-soluble vitamin that helps maintain normal reproduction, vision and immune function.

Beta-carotene (β-carotene) is also known as provitamin A because it is the precursor to vitamin A. It is a plant pigment that occurs naturally in corn, daffodils and mangoes, giving them their golden colour. Scientists created a variety of rice by inserting the gene for β-carotene into rice, giving it a golden colour (see Figure 7.7).

AAP Photo/ Erik de Castro/Reuters

FIGURE 7.7 Golden Rice grains are easily recognisable by their yellow–orange colour, caused by β-carotene (pro Vitamin A).

Golden Rice® is a bioengineered transgenic rice crop with a yellow-coloured grain (endosperm) containing β-carotene. To produce Golden Rice, a gene from the daffodil plant and a gene from a soil bacterium were extracted. These genes were inserted into a plasmid. The recombinant plasmid was then inserted into *Agrobacterium*. *Agrobacterium* reproduced and was then mixed with rice plant embryos. The DNA was taken up by rice embryos, which expressed the two genes when the transgenesis process was successful.

One concern about Golden Rice is the actual effectiveness of the β-carotene after the rice has been cooked. Another concern is cost – it may be less expensive to give those in need supplements. Many argue that the nutrient level of Golden Rice is not significant enough to help alleviate vitamin A deficiency. In addition, there are fears that it will crossbreed with and contaminate wild rice, and, as with other GMOs, worries that transgenic organisms might harm people and be controlled by large corporations more concerned with profit than people.

In December 2019, the use of Golden Rice as a food was approved by the Philippine Government, the first Asian government to approve it. It seems like a massive victory for the Golden Rice Project because they have faced intense activism against the use of this GMO. The World Health Organization lists Filipino mothers as being moderately vitamin A deficient, and children less than 5 years old as being severely vitamin A deficient. The Golden Rice Project predicts that a source of vitamin A will reduce child mortality by 23–34%, and cases of measles by up to 50%, thanks to the immune-boosting effects of vitamin A.

Key concept

Recombinant DNA is used in agriculture to improve yield, quality and productivity. Further research needs to be done to assess the efficacy of transgenic organisms in improving nutrition.

Tolerance to adverse environmental conditions

Crops face a multitude of abiotic stresses in their environment. **Climate change** is arguably aggravating these abiotic stresses. The term 'climate' refers to the statistical description of weather, and it affects the conditions of oceans, land surfaces and ice sheets. It includes consideration of averages, variability and extremes. Climate change is an alteration in the pattern of climate over a long period of time, and may be due to a combination of natural and human-induced causes. The climate change currently being observed is mostly due to human activity, primarily the burning of fossil fuels causing a 40% increase in heat-trapping carbon dioxide in the atmosphere, causing a rise in the average global surface temperature. This has led to extreme weather conditions, which can be the source of adverse environmental conditions. Some abiotic factors, or **adverse conditions**, affecting crops include extreme temperatures, drought, flooding, high salinity, and deficient soil nutrients. Adverse conditions are factors in the environment that affect the survival of

an organism. Monsanto Australia gained approval to grow, sell and use drought-tolerant GM corn in Australia in 2010. This new variety of corn was genetically modified to tolerate cultivation under water-limited conditions. The corn line also contains a commonly used marker gene encoding antibiotic resistance. The drought-tolerance trait is conferred by expression of a single bacterial gene, *cspB*, from the bacterium *Bacillus subtilis*, which encodes cold shock protein B. Cold shock proteins are widely found in bacteria and facilitate adaptation to suboptimal temperatures by preserving protein synthesis. Similar proteins are found in plants and enable them to tolerate various abiotic stresses.

In the Southwest of WA, salt-affected agricultural land currently exceeds one million hectares. This is increasing with time, along with the simultaneous rise of the water table. The implications for WA's agricultural industry are substantial – it results in reductions in fertile land, crop yield and quality, and millions of dollars of lost revenue.

Scientists such as Professor Edward Barrett-Lennard and his team successfully trialled GM wheat and barley tolerant to high saline conditions in 2013–16. Two genes were introduced into the wheat plant to create a transgenic organism; an OAT gene from a common plant species *A thaliana* and the cyanamide hydratase (CAH) gene derived from the soil fungus *Myrothecium verrucaria*. The OAT gene codes for an enzyme that assists growth in elevated salt conditions. The CAH gene codes for an enzyme that is used as a marker to assist in the selection of the GM plants in the laboratory.

One method of introducing the genes into the wheat genomes for transformation is via microprojectile bombardment. Gold particles carrying the genes on linear fragments were shot into wheat embryos using a helium pressure gun. The embryos with the transformed genomes were selected in the presence of cyanamide (a toxin) using the CAH marker gene.

The professor and his team performed trials in high saline areas abiding by strict criteria to reduce the risk of potential hazards. They showed that the gene responsible for the tolerance was of low potential risk as an allergen because the protein products, which are different in structure to known allergens, are found in food that humans already eat safely. The potential for a transfer of the foreign genes into non-target organisms was evaluated. Wheat is predominantly self-pollinating (95%). Although the approximated 5% chance of cross pollination exists, the risks during the trials were removed because it can only cross pollinate with species of the same genus. Due to geographical isolation, there were no closely related species close enough for a transfer.

The GM wheat was subjected to different levels of salinity and compared to the non-GM wheat control group. The GM wheat results were conclusive – where non-GM wheat growth was inhibited by salt levels, GM wheat survived and grew successfully. The future of this line of wheat is uncertain without further funding and support.

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FIGURE 7.8 Dryland salinity causes huge reductions in crop yield.

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FIGURE 7.9 GM wheat can grow in higher salt areas.

Long-chain omega-3 oils in grains

CASE STUDY

Omega-3 oils, especially the long-chain fatty acid docosahexaenoic acid (DHA), are critical for brain and eye development in infants and are an essential part of a healthy adult diet. These healthy oils are not synthesised by humans and thus must be obtained through diet. Land plants do not normally produce DHA. Marine microalgae alone are known to possess the biochemical machinery to synthesise DHA. Fish contain large amounts of DHA because they feed on those algae. Fish are therefore the major dietary source of DHA, but fishing pressure is causing a worldwide decline in fish stocks.

In collaboration with Nuseed and the Grains Research and Development Corporation (GRDC), scientists from CSIRO are working on a project aiming to solve this problem. The Canberra-based team is working towards the production of GM canola that contains long-chain DHA levels similar to those of fish.

After years of research and development, in 2012, the CSIRO team created transgenic *Arabidopsis thaliana* (a commonly used laboratory model plant) by inserting genes (sourced from microalgae) coding for DHA synthesis enzymes into its genome. This was a breakthrough, because these plants then produced seeds with levels of DHA similar to that found in commercially available fish oils. In 2013, the team reported obtaining similar levels of DHA in oil from *Camelina sativa*, oilseed crop.

Since then, the CSIRO team has managed to obtain fish-oil-like levels of DHA from their canola crop in the laboratory. They are continuing to develop GM canola, as its adaptability to a variety of growing regions and its high oil content promise commercial viability. Field trials began in 2014, and canola containing long-chain DHA is now commercially available for fish feed and human consumption.

Questions

1. Learn about omega-3 oils. Find alternative sources of these oils that are currently available, aside from fish. Where would vegetarians source these oils?
2. Think about alternatives. Would it not be simpler to eat algae, rather than make crops that produce DHA? Why do you think this approach was not selected?
3. How would you feel about eating bread and cake enriched with DHA obtained from genetically engineered oil?
4. Why stop there? What genetically engineered crops can you imagine will be developed to produce other essential nutrients or medications?

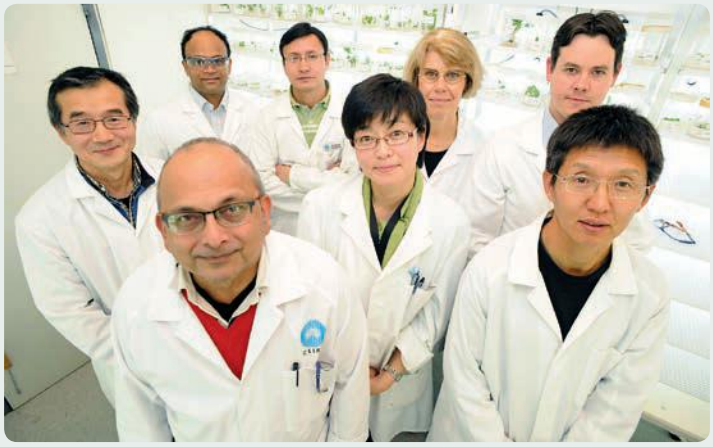

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FIGURE 7.10 The CSIRO research team, left to right, front row to back row: Dr Surinder Singh, Mr Lijun Tian; Dr Qing Liu, Dr Yoko Kennedy; Dr Srinivas Belide, Dr Pushkar Shrestha, Ms Anne Mackenzie and Dr James Petrie

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Genetically engineered animals

In addition to AquAdvantage Salmon®, other genetically engineered animals have been developed. These include cows that produce milk similar to human breast milk, and enviropigs that release less phosphorus in their manure. As well as these examples of farmed animals, laboratory animals (including rats and mice) have been engineered to contain alleles that make them model organisms for studying human disease and for discovering the functions of various genes.

Question set 7.2

REMEMBERING

1 Define:
   - **a** Roundup Ready crop
   - **b** herbicide
   - **c** β-carotene.

2 Describe climate change.

UNDERSTANDING

3 Explain how climate change can increase adverse conditions affecting crops.

CREATING

4 Create a mind map summarising the information provided here about the engineering of transgenic organisms for resistance to pests and diseases, faster growth rate, greater product quality and yield, and tolerance to adverse environmental conditions. Include the name of the GMO, the name of the organism that naturally contains the gene, and a description of the desired trait and the advantage it confers.

7.3 DNA TECHNOLOGIES IN ENVIRONMENTAL CONSERVATION

Right now, a pollution and extinction crisis threatens our living world. Slowing climate change and habitat destruction are our biggest challenges. On 8 November 2019, the Australian state and territory environment ministers endorsed a new approach to biodiversity conservation through 'Australia's Strategy for Nature 2019–2030'. The strategy is supported by a dedicated website: Australia's Nature Hub. Both the Strategy and the Hub are owned and being developed by the Commonwealth, all state and territory governments and the Australian Local Government Association. In addition to protecting biodiversity, the new approach will focus on incorporating adaptation, resilience and natural resource management in our urban, rural and natural (terrestrial and aquatic) environments. **Conservation biology** is the integrated study of ecology, physiology, evolution, molecular biology and genetics with a view to sustaining biological diversity at all levels. It is a broad approach to preserving what remains, and determining the due care and attention needed for protecting it for the future. Biotechnology can be used in environmental conservation for:

- monitoring **endangered species**
- assessing gene pools for breeding programs
- **quarantine**.

Biological diversity levels include genetic, species and ecosystem diversity. The levels are interconnected. This section of the chapter will focus on how biotechnology is used to maintain viable gene pools. A **gene pool** is a collection of all the alleles for all the genes in the reproducing members of a population at a given time. It is the genetic reservoir from which a population can obtain its traits. A large gene pool has large genetic variation and a small gene pool has little variation. A small gene pool is likely to be present in an isolated population, particularly when that population is small. A **viable gene pool** contains sufficient alleles and genes to give enough diversity for survival in a changing

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environment and which avoids loss of fitness as a result of inbreeding. When the environment changes, a population rich in variation will have many alleles present, making it likely that some will suit the new environmental factor. Nature selects those traits that give a survival advantage, through the process known as natural selection. For natural selection to occur there needs to be variation. Preserving high levels of inheritable variation helps to retain a population's current reproductive fitness and also to maintain its evolutionary potential (its capacity to adapt to environmental change over the long term). An important role of conservation is the protection of viable gene pools.

The importance of conservation

Australia has more endemic mammals and reptiles than any other country in the world and more endemic plants than 98% of the world's countries. Endemic species are those that occur nowhere else on Earth. These plants and animals are as much a part of our heritage and identity as significant natural sites, such as Kakadu, Ningaloo, Uluru and the Great Barrier Reef. In addition to contributing to our economy, our native species confer other benefits on the environment. For example, our native bats and birds help control pest insects, spread seeds and maintain our forests. Our plants provide food and shelter for many species, while capturing and storing carbon, combating salinity, keeping riverbanks stable, reducing erosion and improving water quality. Additionally, Australia is home to an agricultural industry that relies in part on natural systems and which feeds us and many other parts of the world.

Conservation is important to protect and ensure the survival of our native species, and it requires careful planning to be successful. Conservation plans should be based on the best available scientific information. Conservation planning to maintain viable gene pools should consider:

- **biogeography**
- **reproductive behaviour**
- **population dynamics**.

Biogeography

To maximise the number of species secured in an ecosystem, there needs to be active protection and restoration of native habitats, mitigation of threats and management of risks to environments. Before an action plan is made, there also needs to be an understanding of the biogeography of the ecosystem.

Biogeography is the study of the distributions of animal and plant species and how those distributions relate to the environment, to the origins of the species and to the changes that have occurred over time. It reveals the spatial organisation of biological diversity. An understanding of the spatial arrangement of each species (the distribution of its population) is vital. The geographical size of an ecosystem, the habitats it contains, and the changes it has undergone over seasonal to geologic time scales all have an impact on its biodiversity. We need to know that nature reserves are large enough and that they have biotic and abiotic factors that are suitable for maintaining a viable gene pool of each individual species. Biogeographical regions have characteristics, such as temperature, elevation, soil types and typical species of plants and animals. If the distribution of a species changes over time, this can help scientists decide whether or not an area needs active protection, restoration or management.

As the climate of a particular area changes, so does its ecology. Modern biogeography often employs the use of Geographic Information Systems (GISs) to understand the factors affecting the distribution of organisms, and to predict future trends in those distributions. Biogeographical studies of invasive species can show how they are dispersing, their likely eventual distribution, and how they might affect the biotic and abiotic factors in the environment, for example, by causing a decline in the population numbers and genetic diversity of other species. Geoscience Australia and CSIRO are working together to generate high-resolution mapping of seabed environments and terrestrial vegetation to better understand the distribution of habitats, which is key to the support of threatened species.

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Reproductive behaviour

Reproductive behaviour is behaviour related to the production and care of offspring, including the establishment of mating systems, courtship, sexual behaviour, fertilisation and raising of young. For animals to reproduce successfully, they must have a favourable situation, often they must undertake particular behaviours leading to the union of male and female gametes, and they must help with the survival and development of the young.

For each species, there is a complex set of behavioural adaptations that coordinate the timing and pattern of reproductive activity. Reproductive behaviour tends to be relatively stereotyped within a species, but diverse among different species, especially if they are distantly related. Reproduction produces viable, fertile offspring that, in turn, will reproduce and thus perpetuate the species. Reproductive behaviour needs to be considered when planning conservation strategies to prevent inbreeding and loss of advantageous alleles, gene pool diversity, and reproductive fitness.

Population dynamics

Population dynamics is the study of the number, gender, age and relatedness of individuals in a population. Population size is directly affected by the number of births, deaths, **immigrations** and **emigrations**. All of these changes can cause a shift in dynamics. But there are many other factors that can affect dynamics, and most of them are complex in their effects.

If a disturbance (such as a bushfire) affects dispersal, then dispersal can become the factor that triggers a major change in population dynamics. Other density-independent factors (such as an infectious disease or tree logging) can cause major changes in population dynamics.

If habitat size and health changes, this can lead to limitations of resources such as food and shelter. These are density-dependent factors that can cause increased competition and predation in a population.

Population growth, density, urbanisation and migration (immigration and emigration) are factors to be considered in population dynamics. A small population usually has a small gene pool. Small populations therefore have a higher risk of losing genetic diversity (especially due to genetic drift), and population size is a key consideration when planning conservation strategies.

Uses of biotechnology in environmental conservation: monitoring endangered species

More than 80% of Australia's mammals and 90% of our trees, ferns and shrubs occur nowhere else on Earth. But since European colonisation, in just over 200 years, more than 130 of Australia's known species have become extinct. The list of those threatened with extinction continues to grow. Australia's threatened species are our responsibility to protect and we all have a role to play. But how do we know if a species' numbers are dangerously low and how would we know if their numbers became stable? Monitoring endangered species is a crucial part of conservation, because it helps scientists identify species threatened with extinction and provides evidence of the effectiveness of conservation strategies. Monitoring data can be used to diagnose the causes of population decline and to measure management effectiveness.

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FIGURE 7.11 The endangered Gouldian finch (*Erythrura gouldiae*)

Factors that are monitored may include behaviour, geographical movements, reproduction, diversity, population size (births/deaths/migration) and population growth. DNA technologies can help monitor endangered species: for example, the Gouldian finch is being monitored with the use of a probe that identifies DNA in water bodies frequented by this critically endangered species. The method for analysing the DNA was developed by researchers from the University of Western Australia and Charles Darwin University.

Damien Cancilla – Spectrum Ecology

FIGURE 7.12 Conservation scientist releasing a northern quoll after taking measurements

Environmental DNA (eDNA) is DNA left behind in an environment by an organism, and it can be used to monitor the platypus (*Ornithorhynchus anatinus*). EnviroDNA is the company using this technology, collecting data about the changing distribution of the platypus over time. As platypuses are difficult to observe in the wild, their eDNA may be the only evidence available for where the vulnerable species is distributed.

The northern quoll is a carnivorous marsupial and can be identified by its distinct white spots. Disconnected populations inhabit the northern part of Australia, including northern WA. Northern quoll populations have declined significantly with land clearing, the increase in the population and distribution of cane toads, and predation by other feral animals such as cats. They are now listed as endangered under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999.

Long-term monitoring of northern quolls is being conducted in the Pilbara to develop better understanding of the factors that are contributing to their decline, and of the factors that may contribute to their survival. Knowing the range boundaries of populations and understanding the barriers to gene flow between them can assist scientists in providing more effective support.

Scats were collected for analysis from the deep gorges the quolls inhabit. DNA analysis and sequencing was conducted by the Department of Parks and Wildlife (now the Department of Biodiversity, Conservation and Attractions), and individuals were identified using DNA profiling. This made it possible to assess the genetic health (diversity) of the populations, which helps guide conservation management. Scientists now know the northern quoll consists of a single genetic population across the arid Pilbara region and can pinpoint areas where laying feral cat baits is likely to have the greatest benefit.

Uses of biotechnology in environmental conservation: breeding programs

Many of the techniques we have discussed thus far have also been employed in efforts to preserve biodiversity and to help manage vulnerable populations. In small populations of animals and plants, there is a risk that closely related (genetically similar) individuals will breed together. The resulting offspring will have an increased risk of deleterious recessive alleles becoming homozygous, making them vulnerable to genetic diseases. This is called **inbreeding depression**. Biotechnologists can use various techniques, including DNA profiling, to selectively breed individuals. This is a common practice in **captive breeding programs (conservation breeding programs)** of threatened species such as the mountain pygmy possum, the Tasmanian devil and the orange-bellied parrot.

Studies have also been carried out to determine the genetic diversity of the gene pool of populations of elephants and cheetahs. These studies will provide information about, and be used to help preserve, the genetic diversity that exists in their populations and thus improve their chances of long-term survival. DNA was extracted from material in their droppings (scats). A major advantage of collecting faecal DNA is that it does not require capturing the animal. This avoids stress to the animal and danger to the researcher.

Saving the Tasmanian devil

The Tasmanian devil (*Sarcophilus harrisii*) once occupied mainland Australia but is now endemic only to Tasmania. Feral animals and farmer culling in the 1800s reduced their numbers greatly. In 1941, they became a protected species and their numbers grew steadily; however, their genetic diversity could not. Tragically, the devil facial tumour disease (DFTD) has killed tens of thousands of them since the mid-1990s. DFTD is one of the rare cancers that can spread as a contagious disease. The mode of spread from an infected devil to a healthy devil is direct, mostly through biting. When a tumour cell is transferred to a healthy devil, most devil's immune systems are not capable of responding effectively. Due to their close contact interactions when they are feeding and fighting for territory and mates, the disease has spread easily in many populations. They are now classified as a vulnerable species.

Maintaining genetic diversity in threatened species is important because it underlies their ability to adapt to changes in the environment and it prevents inbreeding. Genetic methods have been used in Tasmanian devil breeding programs to preserve genetic diversity and minimise inbreeding (the mating of closely related organisms, leading to the expression of rare recessive alleles). Inbreeding can decrease a population's reproductive fitness. Pedigrees, PCR techniques, sequencing and mapping of genomes have helped scientists determine the genetic variation remaining and the relatedness between individual devils. Restriction enzymes have been used to fragment the genome in a systematic way, allowing scientists to sequence sections of the genome. Scientists then compare the sequences of individuals to determine how closely related they are, and store the data on shared databases to assist breeders in selecting the most distantly related individuals to breed. The goal is to maintain or, ideally, increase genetic diversity in the population.

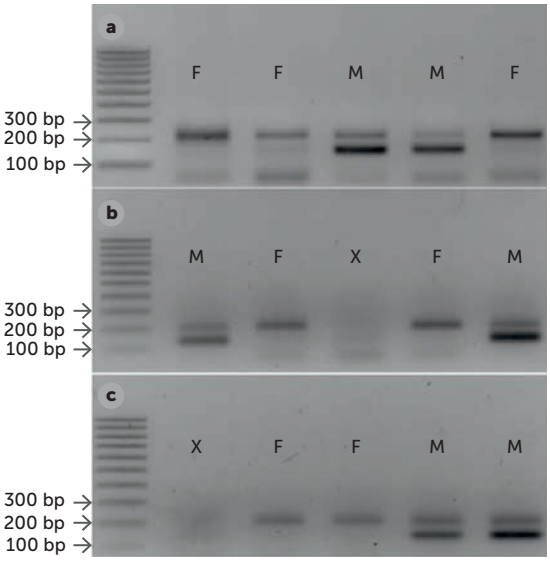

FIGURE 7.13 DNA profiling of Tasmanian devils using DNA from non-invasive sources: tissues **a**, hair **b** and scat **c**.

Minden Pictures/Photographer Suzi Eszterhas

FIGURE 7.14 Tasmania Zoo's precinct is home to a very successful breeding program for Tasmanian devils. The biosecure breeding pens are referred to as 'Devil's heaven'.

Biotechnology has been used to reconstruct more accurate pedigrees to improve the effectiveness of the Tasmanian devil breeding program on Maria Island, a small island just off the coast of Tasmania.

In this study, a multiplex PCR-based assay was used to determine the sex of individual Tasmanian devils. The assay used a new species-specific primer set that amplified a fragment of the *SRY* gene and an autosomal micro-satellite marker as an internal positive control. Sex could be found out using DNA obtained from tissue, hair samples or scats (faeces). This use of biotechnology represents an important step towards effective monitoring and management of Tasmanian devils.

A disease-free population was released onto Maria Island in 2012 and monitored. The Maria Island devils are an **insurance population** (a population brought in from the wild as a safeguard against the species' extinction) in case the disease makes the devils extinct on the main island of Tasmania. DNA analysis showed inbreeding on Maria Island had reduced the genetic diversity to 77%. Computer modelling showed that introducing eight new individuals every 3 years would maintain the Maria Island population above 95% gene diversity for the next 40 years. The Tasmanian devil insurance population breeding program provides a good example of the many ways genetics can inform conservation of a species.

Uses of biotechnology in environmental conservation: quarantine

Quarantine is another active conservation strategy employed in Australia.

Quarantine is the isolation of organisms that have arrived from elsewhere or been exposed to an infectious or contagious disease. They are monitored until scientists confirm the possibility of disease is no longer present. The Australian Quarantine Inspection Service plays an important role in preventing the entry of exotic pests and diseases that could affect plant, animal and human health, and the environment.

The Khapra beetle (*Trogoderma granarium*) is a quarantine-status pest in Australia. Khapra beetle is a regulated quarantine pest in many countries and is currently absent from Australia. Our international trade would be severely impacted if it became established here. It could infest wheat being shipped interstate or overseas. It is more difficult to control than other stored product pests because of its tendency to colonise cracks and crevices, avoiding pesticide treatments. The Khapra beetle is considered the most serious pest of stored grain in the world. It is often confused with a less detrimental pest known as the warehouse beetle. Identification through observation is less accurate than DNA fingerprinting, so the latter is widely used. It involves the extraction of random fragments of beetle DNA that are cut and amplified and tested to see if they are unique to that species. The likelihood of different species having the same DNA fragments is very low. DNA fingerprinting techniques enable biosecurity officers to quickly and accurately identify the Khapra beetle. In the future, developments in the technology are likely to make the process more rapid and more reliable.

FIGURE 7.15 Khapra beetle: one of the world's most destructive pests of grains such as wheat: **a** adult; **b** multiple life stages; **c** larvae

In recent years, a devastating disease of pigs known as African swine fever (ASF) has been spreading globally. There has been a number of outbreaks worldwide, which has increased the risk of the disease entering Australia. There is no vaccine. Pigs can become infected by eating contaminated food, by direct contact with other infected pigs, or from contaminated soil or farm equipment. The disease has a high death rate. The most likely way ASF could enter Australia is through infected pork products that are then fed to pigs. Pork products that have been seized at international airports or mail centres are sent to a laboratory for testing. Testing in 2019 found nearly half the products seized contained ASF virus fragments, up from just 11% in 2018. One way to test for ASF is to use a PCR technique. To prevent the entry and spread into Australia, diagnostic laboratories must have rapid and accurate procedures for specific ASF virus detection, such as PCR.

With the spread of ASF into new countries, a sampling and testing program for ASF in goods surrendered or seized from passengers, or found in mail parcels, was introduced in late 2018. This will help monitor the risk of ASF entering Australia through food products. Using genetic technology, samples can be readily tested for evidence of ASF, and other viruses such as foot and mouth disease (FMD).

The CSIRO Australian Animal Health Laboratory (AAHL) tested samples of pork products (taken from airline passengers and from mail centres) for ASF virus in late 2018, early 2019 and again in September 2019. In the first two periods, the samples were collected in Melbourne and Sydney and in the latter period in Melbourne, Sydney, Perth and Brisbane.

In late 2018, 6 out of 152 samples contained ASF virus fragments. Over the second period, in early February 2019, 40 out of 283 samples contained ASF virus fragments and 2 products tested positive for FMD virus fragments. In September 2019, 202 out of 418 samples tested positive for ASF virus fragments and 3 products tested positive for FMD virus fragments.

FIGURE 7.16 The CSIRO AAHL biocontainment laboratory in Geelong

Inbred koalas

7.1 APPLICATION

Kangaroo Island is host to koalas that were introduced from French Island in Victoria. By studying their DNA and creating a genetic profile for each individual koala, researchers found that the genetic diversity within the Kangaroo Island population was low. With little to no variation between members of the population, they are vulnerable to a rapid population crash.

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FIGURE 7.17 The koala population on Kangaroo Island only has a low level of genetic diversity as it originated from the introduction of a small number of koalas from Victoria.

Are koalas functionally extinct?
Read about the current extinction issues faced by koalas.

Kangaroo Island – a safe haven for koalas
Read about the chlamydia-safe haven for koalas.

Use of recombinant DNA in environmental conservation

Environmental pollution is a major problem that affects biodiversity, public health and ecosystems worldwide. This issue cannot currently be solved using conventional technology, because such methods are expensive, ineffective and time consuming. Conventional methods focus unduly on the separation, rather than the destruction, of contaminants. The use of genetically engineered organisms for **bioremediation** would be an environmentally friendly and cost-effective alternative for the management and remediation of pollutants in contaminated sites. Different types of genetically engineered microbes have been developed through recombinant DNA and RNA technologies, and these have been used to remove heavy metals and toxic substances from contaminated sites. With advancements in biotechnology and microbiology, genetically engineered bacteria are being used in environmental restoration, resulting in bioremediation that is more viable and eco-friendly. Bioremediation is the consumption and breakdown of environmental pollutants by deliberately introduced or naturally occurring micro-organisms. The process is used to treat contaminated water and soil. Usually environmental conditions are altered to stimulate the growth of micro-organisms for the breakdown of a pollutant.

Chlorinated hydrocarbon 1,2,3-trichloropropane (TCP) is a manufactured compound that can be used in varnish remover and other cleaning products. However, it is highly toxic and is carcinogenic. TCP is frequently detected in groundwater because of improper waste disposal. It is a dangerous pollutant because it resists **biodegradation**. Biodegradation is the breakdown of an organic substance by micro-organisms such as bacteria through decomposition. TCP has a higher density than water and is moderately water soluble, so it moves easily into deeper groundwater layers, leading to widespread contamination. It then infiltrates drinking water, becoming a hazard for ecosystems. However, it can be broken down to some degree by the enzyme haloalkane dehalogenase. To address this environmental problem, a gene encoding improved haloalkane dehalogenase (*dhaA31*) was placed into a plasmid and cloned. This engineered haloalkane dehalogenase gene was then introduced into the bacterium *Pseudomonas putida* MC4, to be used in the bioremediation of the pollutant TCP.

Key concept

Biotechnology tools and techniques used in conservation include restriction enzymes, PCR, recombinant DNA, DNA profiling and pedigree analysis.

Question set 7.3

REMEMBERING

1 Define:
   - **a** conservation biology
   - **b** inbreeding depression
   - **c** quarantine
   - **d** biodegradation.

2 State one reason why environmental scientists should consider each of the following factors when planning for conservation:
   - biogeography
   - reproductive behaviour
   - population dynamics.

UNDERSTANDING

3 Describe the difference between a viable and a non-viable gene pool.

4 Differentiate between biodegradation and bioremediation.

REFLECTING

5 Provide a rationale, relevant to your needs as a citizen of Earth, for environmental conservation.

7.4 ETHICAL ISSUES ASSOCIATED WITH TRANSGENIC ORGANISMS

The ability to manipulate and modify DNA carries responsibility. The implications of gene technology and the issues associated with the application of gene technology have to be considered in any decisions about what should or should not be done. GMOs such as crops are theoretically very appealing: farmers get a better yield, spend less on pesticides and herbicides, and don't pour dangerous chemicals into our environment. Yet, how is the modified crop going to affect the ecosystem?

Since the first approval for the commercial sale of GM food in the 1990s (1996 in Australia), there has been significant uptake of its cultivation across the globe. By 2015, nearly 180 million hectares of GM crops had been grown by more than 18 million farmers in 28 countries. Maize, soybeans, cotton and canola are the primary GM crops, and account for 99% of the area devoted to GM production.

As of 2018, GM canola, cotton, safflower and blue carnations are the only GM crops that have been licensed to be grown in Australia by the Office of the Gene Technology Regulator for commercial cultivation in Australia. GM cotton was introduced into the Australian market in 1996 and now represents almost all of the cotton grown here. Monsanto is the trademark owner of the licensed variety, which is resistant to insecticides and other herbicides. In WA, GM cotton and GM canola have been commercially grown since 2008 and 2010, respectively. GM safflower is the most recent addition to the GM crops being grown in Australia, with the first crop being produced in 2018. It is mainly grown in New South Wales, Victoria and South Australia, and is used for industrial oil production and animal feed.

Farming of GM cotton has increased greatly in WA; however, the law governing the growing of GMO and neighbouring crops is not very protective for farmers of non-GMO crops. A court proceeding between a non-GMO farmer and a GMO farmer recently highlighted the need for laws to be developed to protect economic loss for a non-GMO farmer if the non-GMO crops become contaminated. Buffer areas between farms only provide limited protection, due to wind dispersal. Organic standards have been stated as a zero tolerance to GMOs, and the presence of GMOs is prohibited in any segment of the organic food chain.

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The seriousness of potential gene flow into non-target crops became obvious when a GMO farmer was accused of contaminating a non-GM farmer's crops, causing the subsequent loss of his farm's organic certification.

The WA farmer's case (*Marsh v Baxter*) came before the Supreme Court in 2014. Mr Marsh had farmed organic non-GM cereal crops from 2004 and canola from 2000. Adjacent to Mr Marsh's farm was Mr Baxter's farm, on which GM canola seed was grown. Two hundred and forty-five canola swathes (a row of cut grain) containing GM seeds, blew onto Mr Marsh's farm, which led his organic certifier, the National Association for Sustainable Agriculture Australia (NASAA), to decertify 70% of his land in 2010.

Although his land was recertified in 2013, Mr Marsh had suffered economic loss. He took Mr Baxter to court, but lost at trial and again on appeal. It was difficult to demonstrate that the GM material actually came from Mr Baxter's farm, and it was found that Mr Baxter had not acted negligently and could not be held responsible. Additionally, Mr Marsh was not farming canola or any other genetically compatible species at the time. (Therefore, there could not have been transfer of genes.) Although Mr Marsh did not win the case, it did bring to light further ethical issues surrounding GMOs.

Using GMOs may have adverse effects on genetic diversity and on the environment: there may be effects on non-target organisms, more rapid evolution of pesticide-resistant species, and the possibility of **gene flow** from crop species to weed species, resulting in the emergence of **superweeds**.

Effects on non-target organisms

Some transgenic organisms have been engineered to produce toxins deadly to target organisms. For example, Bt cotton has a gene from Bt bacteria inserted into its genome that creates a protein that makes a toxin that affects bollworm. This prevents bollworm from consuming crops. However, there are concerns that other non-target species will be harmed.

The effect of GMOs on non-target species is not yet clear. Some researchers once claimed that the pollen from pest-resistant corn could threaten the monarch butterfly, but these claims have now been disproved. However, further testing of pollinating, non-target insects such as butterflies, bees and water fleas is currently being conducted. The insects being tested include species feeding on the transgenic Bt crops that produce the deadly toxin.

The World Health Organization is not opposed to GMOs, but they do raise concerns that should be addressed. Gene transfer from GM foods to cells of the body or to bacteria in the gastrointestinal tract would cause concern if the transferred genetic material adversely affects human health. This would be particularly relevant if antibiotic-resistance genes, used as markers when creating GMOs, were to be transferred. Although the probability of transfer is low, the use of gene transfer technology that does not involve antibiotic-resistance genes is encouraged.

Additionally, they are concerned about **outcrossing**. Outcrossing is the migration of genes from GM plants into conventional crops or related species in the wild. Outcrossing and the mixing of GM crops with crops derived from conventional seeds may have an indirect effect on food safety and food security. Cases have been reported in which GM crops approved for animal feed or industrial use were detected at low levels in products intended for human consumption. Several countries have adopted strategies to reduce mixing, including a clear separation of the fields within which GM crops and conventional crops are grown.

More rapid evolution of pesticide-resistant species

Studies in India have demonstrated the evolution of toxin resistance in aphids and mealy bugs, resulting in the inefficacy of GM corn.

Roundup Ready crops became popular in the mid-1990s. Farmers bought seeds with the genetic modification for tolerating glyphosate (a chemical used in Roundup Ready). Glyphosate is the primary active ingredient in this brand of commercial weedkiller. After decades of use, some detrimental effects have been observed. Some invasive plants (weeds) have also developed resistance through

GM for good
Watch this video from the ABC's science show 'Catalyst'.

Genetic Literacy Project
Read current news about genetics and biotechnology here.

natural selection, a mechanism of evolution. This has led to farmers using more of the chemical to kill weeds to protect crops. Research conducted in the south-west of the US found that GM crop farming uses 25 per cent more herbicides than non-GM farming.

Another concern is that transgenic organisms themselves, with herbicide resistance engineered, may evolve quickly with the limiting factor(s) for growth removed. If an adverse condition was removed from the environment of a herbicide-resistant crop, for example, the transgenic crop growth rate could be much higher, resulting in the transgenic organism evolving into a pest. The GMO pest could then outcompete native plants or other crop plants and deplete the soil of nutrients.

Key concept

As crops and weeds develop resistance to herbicides, more herbicides are applied by farmers to control weeds. The increase in herbicide spraying has sped up the evolution of resistant weeds by natural selection, and has also increased the level of pollution entering ecosystems.

Science Photo Library/Bruno Petriglia

FIGURE 7.18 Herbicide-resistant horseweeds

Emergence of superweeds

What about the spread of modified genes into the surrounding environment? This depends on how the plant is pollinated. Plants such as cotton are usually wind pollinated. This could lead to the spread of modified plants to other nearby crops belonging to farmers who may not want to use this type of crop because of the unknown long-term effects or its poor acceptance among some consumers.

Gene flow may occur from transgenic crop plants to other species via wind or contaminated tools. This means the introduced gene may be transferred. The introduced gene may have been selected for herbicide resistance, pest resistance or drought resistance. The newly modified species may be transformed to start expressing the gene, assisting it to increase its growth rate and become a pest/weed. Farmers may be unable to control the growth of such a weed. This type of weed is known as a superweed.

Canola is typically alternated on a 2-yearly cycle with a cereal crop such as wheat. Multiple-resistant oil seed rape appears as a weed in the following year's crop, especially around field margins where seeds spilled during harvest can gather. A Canadian study found that these plants contained resistance genes from up to three GM varieties – so-called gene stacking. Farmers were then forced to resort to a different and much more persistent herbicide, 2,4-D, to control them.

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Reduction in genetic diversity in GMO crop plants

The use of transgenic crops may lead to a loss of genetic diversity. Usually, farmers plant a single crop that has a desired gene inserted into its genome. The crop is a single strain of the plant species and is therefore a **monoculture**. The favourable characteristic may help it survive a specific factor in the environment such as drought. However, the gene pool is limited for surviving other changes in its environment. If a new pest or disease emerges, there may not be a suitable allele in the monoculture's gene pool that will enable it to survive. The lower a crop's genetic diversity, the higher its susceptibility to environmental change. This is an important issue, because climate change is causing new environmental factors to emerge.

Arguments for and against transgenic organisms

Another concern raised by consumers relates to a belief that an increased allergenic effect might be induced when combining genes from two organisms. If a gene that produces a protein from one plant is introduced into a second plant to give it a desired trait, there is a chance that the introduced transgene might cause an allergic reaction in some people.

The application of biotechnology is fraught with controversy: there are arguments for and against, and the merits of each application have to be evaluated from a number of perspectives. Scientific, industrial, commercial and governmental interests have to be examined, along with the views of the rest of society. Gene technology is costly, and who does it benefit? We have powerful tools available to us, capable of changing the course of life, and their use needs to be debated.

TABLE 7.1 Arguments for and against biotechnology

FOR	AGAINST
Biotechnology is natural; genetic engineering has existed for years; for example, farmers breed specific cattle to achieve the desired traits. Biotechnology is simply an extension of this process.	Biotechnology is not natural; selective breeding only involves individuals from the same species, whereas biotechnology can mean transferring genes across species, which rarely happens naturally.

Modifying plants and animals and releasing them into the environment raises the possibility of them affecting organisms in ecosystems. Table 7.2 presents some of the arguments for and against the use of biotechnology in terms of its environmental effects.

TABLE 7.2 The effects of biotechnology on the environment

POSSIBLE POSITIVE EFFECTS ON THE ENVIRONMENT	POSSIBLE NEGATIVE EFFECTS ON THE ENVIRONMENT
Herbicide-resistant crops could be made resistant to a herbicide that is not very toxic to the environment. This might enable farmers to use a less toxic herbicide, rather than use one that may be more damaging to the environment.	Herbicide-resistant crops may encourage farmers to use more herbicide on their crops, which could potentially be more damaging for the environment.
Researchers, so far, have found that there is little transfer of genes between species.	There may be gene transfer between closely related species; weeds may become resistant to the herbicide.
	We may not know what gene transfers have occurred.
	We may not know what transgenic organisms have escaped into the environment.

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Proteins are being produced for use by humans, using other animals or bacteria, and plant crops are produced that have a higher yield. Fruit such as bananas and tomatoes are being modified so that they don't bruise on the way to market or ripen too quickly. With these advancements come another set of issues for people who are going to eat the GMOs (Table 7.3).

TABLE 7.3 Arguments for and against genetically engineering foods with respect to public health

ARGUMENTS FOR	ARGUMENTS AGAINST
Biotechnology can vastly improve the health, nutritional value and growth capacity of agricultural species, and therefore greatly help to combat a global food crisis and benefit public health.	Selective breeding has provided us with crop improvements in the past and can be a source of steady improvement in crop quality.
There are strict guidelines that aim to ensure all genetically engineered food is as safe as non-genetically engineered food. The genetic code is common in all living species.	The long-term effects of genetic modification of crops are essentially unknown.

With the increased use of biotechnology, governments of the world have an obligation to keep the public informed about issues that are important to them. Many issues have emerged from the use of genetic engineering. Advisory committees have been set up worldwide for the purpose of alerting the authorities to any risk factors and to ensure guidelines are consistent worldwide.

SCIENTIFIC LITERACY

Superweed outbreak starts herbicide race

In the US' Farm Belt, superweeds such as pigweed that are immune to what had previously been the most effective weedkiller, Roundup, are taking over prime crop fields. This has caused large farming businesses to use greater amounds of very harsh older herbicides.

Monsanto, the company that makes Roundup also sells seeds for plants that are not affected by Roundup, including corn and cotton. This means that farmers can spray these crops without worrying about it harming them. These `Roundup-ready' crops now account for 80% of all the corn grown in the US.

Roundup proved so effective that many of the older herbicides that damaged both weeds and crops ceased to be used. But these new superweeds that are proving resistant to Roundup have seen chemical companies start to increase production on the older herbicides to try to wipe out the superweeds.

In addition, the chemical companies are creating new crop varieties that are resistant to the old herbicides. This will mean that farmers can spray the herbicides on to their crops without worry, rather than be very careful about where they spray the herbicide.

Questions

1. Glyphosate is the active ingredient of Roundup. Research and explain why Monsanto initially chose to engineer crops that are resistant to glyphosate rather than to another chemical.
2. Evaluate the return to older, more powerful herbicides as a long-term solution to this problem.
3. Suggest how pigweed may have acquired glyphosate resistance.
4. Given the information provided above, discuss the benefits of herbicide-resistant crops to farmers and how this balances with the benefits to the company that produces them.

Question set 7.4

REMEMBERING

1 Explain the term superweed.

UNDERSTANDING

2 Differentiate between target and non-target organisms in the context of GM crops.

REFLECTING

3 Summarise the events that led up to the Marsh vs Baxter case. What are some of the issues this case highlighted for the farming industry?

4 'The problem with producing herbicide-resistant crops is that weeds also become resistant and farmers have to use more herbicides to control the weeds.' Discuss why this problem is an issue for farmers and the environment.

5 Draw a table and make a summary of the benefits and risks of transgenic crops.

7.5 EMERGING TECHNOLOGIES

Biotechnologists are investigating a number of new techniques that use DNA, including cloning and stem cell therapy. In the future, these may be used on their own or in conjunction with other processes for benefits in both medicine and agriculture.

Cloning

Cloning is the process of making an identical copy of an original. In biology, it is used in two contexts. First, there is cloning a gene, which involves using recombinant technology: a gene is extracted from one organism and then inserted into a bacterium, where it is reproduced many times for various uses and further study.

Second, there is biological cloning, which involves cloning an entire organism: reproductive cloning. Cloning can make it possible for cattle or sheep with desirable characteristics, such as high milk production or fine wool growth, to be produced more rapidly than through the normal cycles of reproduction and selection. It is achieved by embryo splitting or by nuclear transfer.

Embryo splitting

In embryo splitting, egg cells are removed from the donor female and fertilised in vitro (i.e. in tissue culture) by sperm from the male. After the zygote has divided, the coat around the two cells that promotes cell division is removed and the two cells are separated. Each cell is then given an artificial coating that promotes cell division. Embryos that have just begun to differentiate, called blastocysts, are implanted into surrogate mothers. The individuals are genetically identical; they are like identical twins but are carried by different surrogate mothers.

Nuclear transfer

The process of cloning by nuclear transfer came to prominence when Dolly the sheep was cloned in 1996. Nuclear transfer involves removing mature donor somatic cells from a mature animal and a recipient egg cell from another mature animal of the same species (Figure 7.19 on page 238). The donor cells are cultured in a nutrient medium before being inactivated, and the nucleus of the recipient egg cell is removed.

The intact nucleus from a donor cell is fused with the hollow egg from the recipient cow by an electrical impulse. The new single-celled embryo is cultured for about a week, then cell division is activated and the developing blastocyst is surgically implanted into the surrogate mother. The offspring is genetically identical to the nucleus donor.

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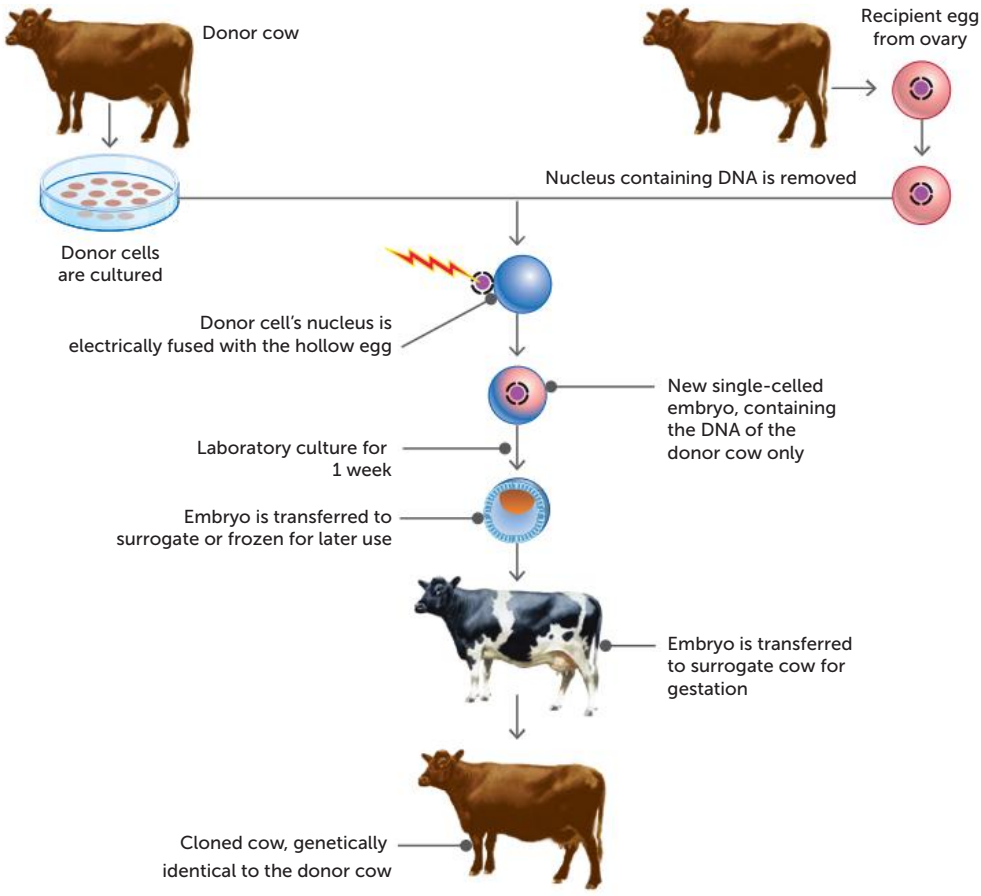

FIGURE 7.19 Using nuclear transfer to clone a cow from a donor cow

Cloning using nuclear transfer has not been without controversy. The success rate of live births is low, and many of the offspring suffer from severe deformities. For these reasons alone, the scientific world is almost universally opposed to experimenting with reproductive cloning of humans.

Stem cell research (optional extra study)

Most of the cells of our body, for example blood, liver, brain and nerve cells, are specialised to perform particular functions. These specialised cells have become differentiated by following a particular developmental pathway, and for them there is no turning back. In contrast, stem cells are undifferentiated cells that have the potential to develop into many different kinds of cell. Unlike differentiated cells, stem cells also have the capacity to keep dividing and renewing themselves. These two characteristics make them ideal for cell-based therapies that aim to replace tissues that have degenerated or been damaged, such as in Parkinson's disease, diabetes, heart failure and spinal injuries. Stem cells may prove useful in the development of new methods for gene therapy, or for researching early stages in human development, and for understanding cell differentiation and function. Research into stem cells may also provide answers as to why cells become cancerous and how this may be prevented. When grown in culture, stem cells can be useful for testing drugs safely before they are trialled in humans, or for screening pesticides for the effects of potential toxins before they are used in the environment.

There are two main types of stem cell: embryonic and adult. Embryonic stem cells are derived from a 4–5-day-old embryo. They have the ability to form virtually any type of cell found in the human body, and are therefore said to be pluripotent, which makes them particularly attractive for cell-based therapies. Embryos are obtained from parents who have completed fertility treatment

and have consented to donate excess embryos to research. However, to obtain the stem cells requires the destruction of an embryo, which raises significant ethical issues, and governments have had to ensure that there are strict regulations for controlling the use of this technology. Embryonic stem cells grown in culture (Figure 7.20) also have a tendency to form tumours called teratomas, which may limit their usefulness.

Adult stem cells are undifferentiated cells found among differentiated cells in a tissue or organ after birth. They are multipotent and can give rise to a more limited range of cell types, which may limit their usefulness in cell-based therapies. However, they do not form teratomas and therefore may be safer to use. Adult stem cells also have the advantage of avoiding the ethical issues associated with the use of embryonic cells, and tissues generated from these cells can circumvent problems with transplant rejection if they are derived from the person who is to receive the stem cell therapy.

Under specific circumstances, differentiated cells can also be made to divide again by artificially inducing the expression of specific genes. These are called induced pluripotent stem cells, and they also can be used as a source of stem cells. Considerable research is being undertaken to try to understand how to make and use induced pluripotent stem cells.

There are pros and cons for using the various types of stem cells, and research is continuing. Other research is also looking into the obtaining of stem cells from umbilical cords and placentas.

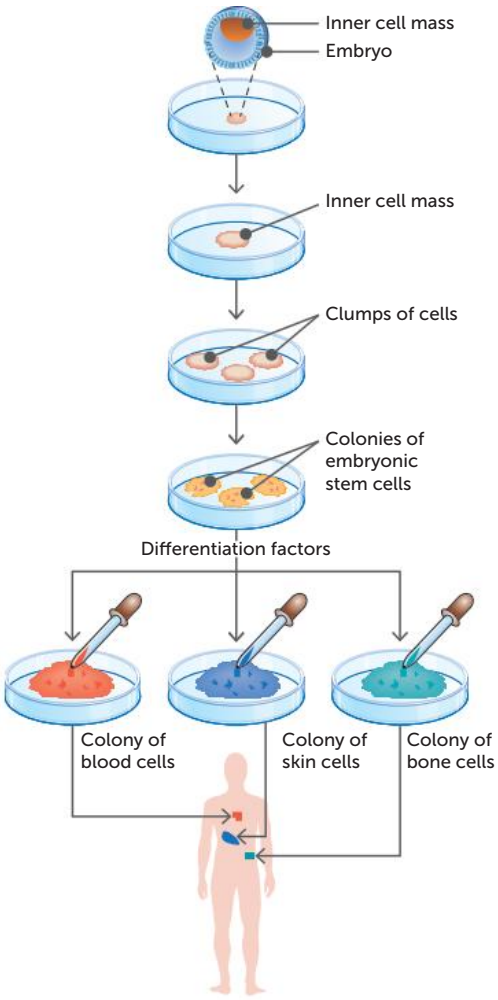

FIGURE 7.20 Embryonic stem cells from humans can be cultured and made to differentiate into any type of human tissue.

Key concept

New DNA technologies, such as cloning and stem cell research, provide hope for solving the current issues with DNA technology as well as hope regarding future applications.

Question set 7.5

REMEMBERING

1 Define:
   - **a** cloning
   - **b** stem cell.
2 Recombinant DNA technology is a form of which type of cloning?
3 List the two techniques used in biological cloning.

UNDERSTANDING

4 Describe how genetically similar a clone of you would be to yourself.
5 Summarise the differences between embryonic stem cells and adult stem cells.
6 Explain why stem cells may be useful for treating diseases such as Parkinson's disease and diabetes.

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CHAPTER 7 ACTIVITY

7.1 ACTIVITY

Speciation and conservation: the eastern barred bandicoot

Getty Images/Steve Kaufman

FIGURE 7.21 The eastern barred bandicoot (*Perameles gunnii*)

The eastern barred bandicoot (*Parameles gunnii*) belongs to the marsupial family Peramelidae. It is small (body about 300 mm, tail 200 mm), grey-brown in colour and has four pale stripes or 'bars' on its hindquarters. It has three claws on its front feet, which it uses for digging, while the back feet are long, similar to those of a kangaroo.

Populations of the eastern barred bandicoot were once common over a wide area of south-western Victoria, but numbers were reduced dramatically in the 1900s. The Victorian population of the eastern barred bandicoot got to the brink of extinction in the late 1980s. This was the result of widespread loss of habitat (from clearing of woodlands, growing of exotic pasture grasses, and grazing by domestic stock) and the introduction of cats and foxes.

Conservation plans for the eastern barred bandicoot depended heavily on how its populations were classified. A subspecies is a level of classification below species. It refers to a race of a species that is fairly permanently geographically isolated from other populations of the species, and which may in future diverge to become a separate species. Because of the relatively healthy eastern barred bandicoot populations in Tasmania, the eastern barred bandicoot was not regarded as an endangered species. If the Victorian population were classified as a different species, or subspecies, however, then it could be recognised independently for conservation purposes.

A number of studies were conducted on the Victorian and Tasmanian populations. The bandicoots were trapped, small blood samples taken, and the animals released immediately where they were caught. The blood was snap frozen, and later a DNA fingerprint was taken by analysing the genomic

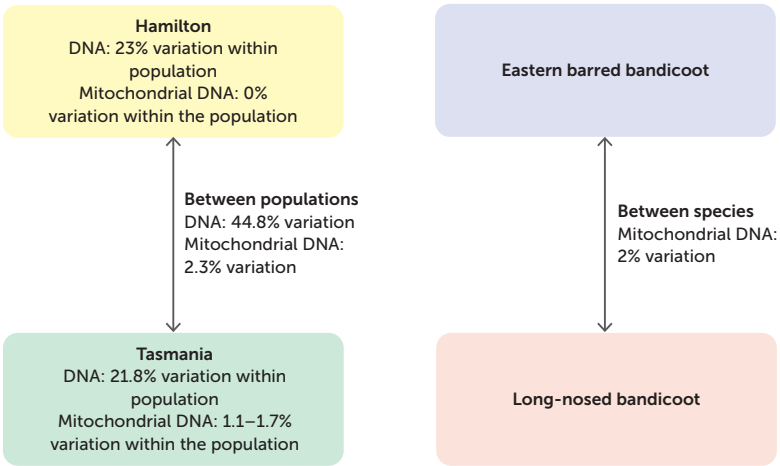

FIGURE 7.22 DNA variability in different populations of eastern barred bandicoots. A 2% variation is the average difference between subspecies of a mammal species.

variable nucleotide tandem repeats (VNTRs). The average percentage difference in the VNTRs within the populations around Hamilton was found to be about 23%, and for those in Tasmania it was 21.8%. The average percentage difference between the Hamilton and Tasmanian populations was 44.8%.

Further testing was done using mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis. This revealed a 0% nucleotide variation within the Tasmanian populations and a 1.1–1.7% variation within the Victorian populations. The percentage variation between the Victorian and Tasmanian populations was 2.3%. A variation of 2% is the average difference between subspecies of a mammal species.

There was no doubt that the two populations had diverged to some extent, due to geographical isolation (see 'Divergent evolution', page 276). The two populations are now considered separate subspecies. This is vital to how the conservation of these two populations of eastern barred bandicoots can be managed.

Biologists currently use a variety of species concepts, all of which are based on the theory of evolution. The biological species concept defines a species as a reproductive community of populations that occupies a specific niche in nature. The identification of species often uses data from genetic analysis, and DNA fingerprinting is often used to determine which groups are related – that is, share a gene pool – and which aren't. A species defined according to this concept would be the smallest group of organisms that share a common ancestor not shared by any other organism.

The Australian Government, through the Department of Sustainability, Environment, Water, Population and Communities, has now listed two subspecies of *P. gunnii*. The following is an excerpt from the listing for the eastern barred bandicoot.

> Scientific name: *Perameles gunnii* unnamed subspecies
> Common name: eastern barred bandicoot (mainland)
> The genetic diversity, as measured by the variable number of tandem repeat markers and mitochondrial DNA restriction fragment length polymorphisms, among specimens from Hamilton, Victoria was greater than that found in widespread populations of the Tasmanian subspecies (*Perameles gunnii gunnii*). The justification for considering the mainland form to be distinct is based in part on morphological comparisons of island and mainland forms, and that mtDNA data indicated separation of 270 000–620 000 years ago.

A long-term captive breeding program, followed by a collaborative genetic rescue program, was undertaken, because the genetic variation within the mainland subspecies was found to have been depleted, threatening their long-term persistence. Male bandicoots from genetically diverse Tasmanian populations were brought across to breed with Victorian females at the Mount Rothwell Conservation and Research Centre. The program was successful and the scientists involved celebrated a 200% increase in genetic variation in that population. A successful captive breeding program commenced in 1991 has now established a population at Mt Rothwell. Other releases at Hamilton, Churchill Island and Phillip Island are looking promising.

Aim

To investigate speciation in the eastern barred bandicoot and relate this to conservation approaches

Questions

1. Which species definition could be used to justify classifying the two populations as separate subspecies?
2. How might the recognition of two separate subspecies by the Australian Government help in re-establishing the eastern barred bandicoot in mainland Victoria?
3. In your opinion, could losing the mainland subspecies of the eastern barred bandicoot affect the long-term survival of the species as a whole? Explain.

Coming to the genetic rescue of our endangered marsupials
Learn more about genetic rescue at this site.

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WS
Chapter 7 Activity sheet

CHAPTER 7 SUMMARY

- DNA identification technologies are applied in agriculture to identify genes in crops and animals that offer increased yield, quality and productivity.
- DNA identification technologies are applied in environmental conservation to maintain genetic diversity. Specific applications are:
  - monitoring of endangered species
  - assessing gene pools for breeding programs
  - quarantine.
- Recombinant DNA technology is being applied in agriculture as genes that increase yield can be identified, extracted and inserted into crops.
- Transgenic organisms have been engineered for:
  - resistance to pests and diseases
  - faster growth rate
  - greater product quality and yield
  - tolerance to adverse environmental conditions.
- Recombinant DNA technology is being applied in environmental conservation: for example, the introduction of GM micro-organisms to polluted areas for the biodegradation of pollutants.
- Conservation breeding programs require careful planning and should consider the following factors:
  - biogeography
  - reproductive behaviour
  - population dynamics.
- Using transgenic organisms may have adverse effects on genetic diversity and on the environment, including:
  - effects on non-target organisms
  - more rapid evolution of pesticide-resistant species
  - the possibility of gene flow resulting in superweeds
  - reduced genetic variation.
- Australia has strict laws governing the commercialisation of GMOs; however, the protection of non-GMO crop farming remains an issue.
- Emerging technologies are being further developed, including cloning and stem cell research.

CHAPTER 7 GLOSSARY

Adverse conditions Factors in the environment detrimental to the survival or growth of an organism

Agriculture The science and management of growing crops and livestock, including cultivation of the soil or other medium

Beta-carotene (β-carotene) A plant pigment that can be converted to vitamin A after consumption

Biodegradation The breakdown of an organic substance by micro-organisms (such as bacteria or fungi) through decomposition

Bioengineering The combination of biology and engineering tools to create a usable product, such as a transgenic organism

Biogeography The study of the distributions of living things over a geographical area and how those distributions have changed over geologic time

Bioremediation Consumption and breakdown of environmental pollutants by deliberately introduced or naturally occurring micro-organisms; the process is used to treat contaminated water and soil

Captive breeding program (conservation breeding program) A breeding program that aims to maintain or increase genetic variation in a population of an endangered species in order to avoid extinction

Climate change The current climate change occurring on Earth encompasses increasing global average air and ocean temperatures, rising global sea levels, long-term sustained widespread reduction in snow and ice cover,

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and changes in atmospheric circulation, ocean circulation and regional weather patterns, which in turn influence seasonal rainfall conditions. The current change is thought to be mostly due to human activity, primarily the burning of fossil fuels

Conservation biology The integrated study of ecology, physiology, evolution, molecular biology and genetics with a view to sustaining biodiversity at all levels; a broad approach to preserving what biodiversity remains, and determining the due care and attention needed for protecting it for the future

Emigration Leaving a country or region

Endangered species A species threatened with extinction

Gene flow The transfer of genes from one population to another; in relation to agriculture, this could mean from GMO crops to other species

Gene pool A collection of all the alleles for all the genes in the reproducing members of a population at a given time; it is the genetic reservoir from which a population can obtain its traits

Genetically modified organism (GMO) See **transgenic organism**

Herbicide Substance used to control or kill weeds, ideally leaving a crop unharmed

Immigration Moving to a new country or region

Inbreeding depression Occurs in small, isolated populations of animals and plants that are closely related, and thus genetically similar; the offspring have an increased risk of deleterious recessive alleles becoming homozygous, causing genetic diseases

Insurance population A population brought in from the wild as a safeguard against a species' extinction

Marker-assisted breeding Selection of seeds for a breeding program by plant scientists that involves checking the seeds for DNA markers that are associated with beneficial traits

Monoculture The practice of growing a single strain or variety of crop in a particular area

Outcrossing The migration of genes from GM plants to conventional crops or wild species

Population dynamics The study of the number, gender, age and relatedness of individuals in a population; population growth, density, urbanisation and migration (immigration and emigration) are factors considered in population dynamics

Quarantine A period of isolation serving to prevent the spread of a contagious disease; suspected cases are isolated from local, susceptible populations until at least the incubation period is finished, clinical signs and symptoms have passed and a scientist confirms the suspected pathogen is no longer present

Recombinant DNA technology Tools and techniques used to transfer a gene from a cell of a member of one species to the genome of a different species

Reproductive behaviour Patterns of animal behaviour related to the production and care of offspring, including the establishment of mating systems, courtship, sexual behaviour, fertilisation, and raising of young

Roundup Ready® crop Crop tolerant to the herbicide called Roundup Ready, which contains the active ingredient glyphosate

Superweed A species of plant, transformed by a gene from a GMO to increase its growth rate, disease resistance or tolerance of environmental limits, that has become difficult to control; it is able to outcompete native or crop species and has become a significant weed

Transformation The process by which DNA is taken from one organism and inserted into another organism, usually of another species, to obtain a desired characteristic

Transgenic organism An organism that has been modified by incorporating into its genome a piece of foreign DNA

Viable gene pool A minimum collection of alleles and genes that have enough diversity for survival in a changing environment, even when limited to inbreeding within the population

Vitamin A A fat-soluble vitamin that helps maintain normal reproduction, vision and immune function

Yield A measure of production of a crop per unit area of land cultivation, and the seed generation of the plant itself; farmers calculate the yield by counting the number of grains in at least 10 heads or pods and calculating the average number of grains per head or pod

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CHAPTER 7 REVIEW QUESTIONS

Remembering

1 Describe an example of the application of a transgenic organism in agriculture to obtain an increase in yield.

2 List three endangered species involved in a captive breeding program.

Understanding

3 Some critics of GMOs are concerned about potential cross-pollination of GMOs with organic crops, because pollen, a male gamete, can be carried by the wind to neighbouring fields. Cross-pollination does occur naturally in the wild and produces hybrid plants. For cross-pollination of a GMO to succeed with another species, the species needs to be similar. Explain what this means.

4 PCR can be applied both in identification and in recombinant biotechnology processes. Describe its role in both.

Analysing

5 One concern raised about GM crops is that some traits may be 'too advantageous'. Explain why this may be a concern.

The following relates to Questions 6 and 7. GM salmon (AquAdvantage salmon®) have been bred to reach market weight in half the time taken by their non-GM form. Many people are concerned about what would happen if they escaped into the wild. Some believe that engineered salmon would grow at a faster rate than non-GM salmon in a natural environment, which is what occurs in a hatchery environment. The results of one study, performed in a similar transgenic salmon, are summarised in the graph in Figure 7.23.

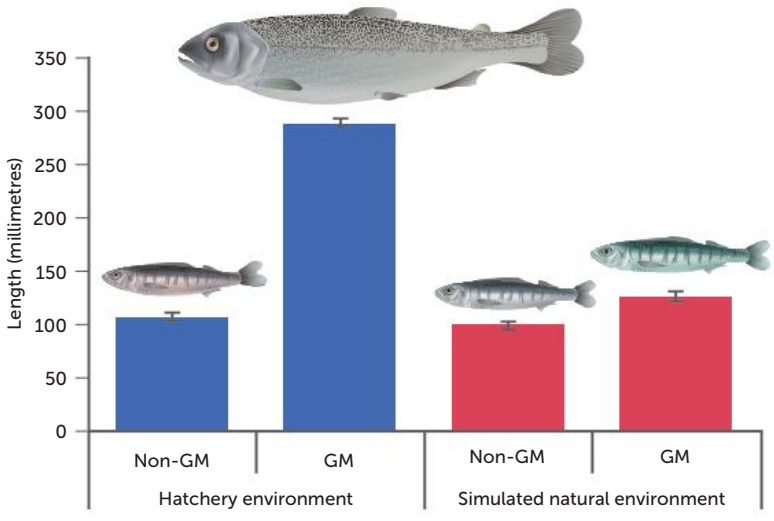

FIGURE 7.23 Length of non-GM and GM salmon bred in different environments

6 Using the information in the graph, determine the following:

a the average length of a GM salmon in a hatchery environment

b the length of a GM salmon divided by the length of a non-GM salmon in a hatchery environment

c the length of a GM salmon divided by the length of a non-GM salmon in a simulated natural environment.

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7 Select the correct word in the following conclusion and rewrite the statement.
It was shown that the increase in the growth rate of the GM fish compared with the wild fish in the simulated natural habitat was greater/less than that observed in the hatchery tank.

Evaluating

8 Describe the potential impact escaped GM fish might have on non-GM fish.
9 Describe three potential benefits of GMOs.
10 Discuss three possible harmful effects of genetic modification.
11 State the purpose of a captive breeding program.
12 Describe the role of DNA profiling in a captive breeding program.
13 Due to habitat loss and bushfires, koala distribution has become fragmented. Isolation of populations has led to inbreeding. Explain the impact of inbreeding on a population.
14 State four ways in which agriculture has been improved through the use of biotechnology.

Reflecting

15 Discuss the potential benefits of the application of biotechnology in biology.

PRACTICE EXAM QUESTIONS

1 Biogeography is the study of:
A population dynamics
B reproductive behaviour
C infectious diseases
D species' distributions.

[Q23 2018 SCSA]

2 The following list shows the main steps in a genetic engineering experiment, in no particular order.

Step A. Enzyme cuts source DNA at specific sites.
Step B. Bacterial cells with recombinant plasmid reproduce.
Step C. Source DNA is combined with a plasmid.
Step D. Bacterial cells with recombinant plasmid are selected.
Which of the following lists these steps in the order in which they occur in the experiment?
A A → C → D → B
B B → D → A → C
C C → B → A → D
D D → C → B → A

[Q25 2018 SCSA]

Use the following information to answer Questions 3 and 4.

A biologist measured the amount of genetic diversity in five populations of the Australian platypus. The amount of genetic diversity in each population is indicated by the diversity index. Values of the diversity index range from 0 (no diversity) to 1 (maximum diversity).

POPULATION	DIVERSITY INDEX
Central Victoria	0.597
North-western Tasmania	0.606
King Island	0.032
Kangaroo Island (wild)	0.419
Kangaroo Island (sanctuary)	0.431

3 The mean value of the diversity index in the five platypus populations is:
A 0.346
B 0.417
C 0.236
D 0.504.

[Q22 2016 SCSA]

4 On the basis of the information in the table, which of the following platypus populations is at the greatest risk of extinction due to genetic factors?

A Kangaroo Island (wild)
B Kangaroo Island (sanctuary)
C north-western Tasmania
D King Island

[Q23 2016 SCSA]

5 *Agrobacterium* is commonly used in the production of transgenic plants (its capacity to cause disease is deactivated first). Outline the role that *Agrobacterium* plays in the production of transgenic plants and explain why it is well suited to this role. (4 marks)

[Q34e 2019 SCSA]

6 Explain how biotechnology can be used to determine the father of a litter. (4 marks)

[Q33e 2018 SCSA]

7 Explain how the use of transgenic crop plants may have adverse biological effects. (10 marks)

[Q37b 2019 SCSA]

8 Discuss why populations with reduced genetic diversity face an increased risk of extinction and how biotechnology can be used to reduce this risk. (10 marks)

[Q37b 2018 SCSA]

9 Chymosin is an enzyme produced by nursing calves to assist with the digestion of milk. Humans also use chymosin to make cheese. Traditionally, chymosin for cheesemaking was obtained from the stomach of calves that had been killed for their meat. It is now obtained from genetically modified micro-organisms. Describe the advantages of obtaining chymosin for cheesemaking in this way. (4 marks)

[Q36a 2017 SCSA]

10 In making conservation plans to maintain viable gene pools, why do biogeography, reproductive behaviour and population dynamics need to be considered? (10 marks)

[Q36b 2017 SCSA]

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8 EVIDENCE FOR THE THEORY OF EVOLUTION

CHAPTER 8 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Preserved bones and tracks are two types of fossils. Can you describe other types of fossils?
2 Do you know what a theory is? Can you explain why evolution is described as a theory?
3 Bioinformatics is a science that is integral to most aspects of biological research: sequence analysis, next-generation sequencing analysis, working out evolutionary relationships and phylogeny. But do you know what it involves?

SCIENCE UNDERSTANDING

» life has existed on Earth for approximately 3.5 billion years and has changed and diversified over time
» evidence for the theory of evolution includes
  – comparative genomics (molecular evidence)
  – the fossil record
  – comparative anatomy and embryology
» evolutionary relationships between groups can be represented using phylogenetic trees

SCIENCE AS A HUMAN ENDEAVOUR

» technological developments in the fields of comparative genomics, comparative biochemistry and bioinformatics have enabled identification of further evidence for evolutionary relationships

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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8.1 LIVING THINGS CHANGE AND DIVERSIFY

Life has existed on Earth for approximately 3.5 billion years and has changed and diversified over time. The life forms we see on Earth today are incredibly varied and beautiful. In addition, many of the characteristics of these diverse life forms seem to help the various **species** to survive in their unique environmental conditions.

The diversity has come about through many changes. Changes that occur in a population of living things accumulate over generations (over a long period of time), because they are inheritable and transform a population's gene pool. This type of change is known as **evolution**. Evolution is the process of cumulative, inheritable change in a population over many generations.

Information on Australian species has traditionally been housed in museums, universities, and government departments and organisations. In 2006, however, a collaboration between CSIRO, Australia's museums and herbaria, universities, and the Australian Government commenced development of the Atlas of Living Australia (ALA), a national project focused on making biodiversity information accessible and usable. As part of this project, CSIRO has created a free online database to support the research and monitoring of Australia's biodiversity, showcasing Australia's amazing variety of animals and plants. They built the tool so that all Australians interested in monitoring and maintaining our biodiversity can collaborate, accessing and sharing data quickly.

Atlas of Living Australia
Click on the 'Community and Schools' tab. Then click on the 'Explore Your Area' option. Type in your postcode and view some of the diverse species that inhabit your local area.

Australia has environments ranging from tropical rainforest to desert to coastal to alpine. As a result, we also have a wide range of unique native species. Figure 8.1 shows a selection of animals native to WA. You can observe, and you may also have some previous knowledge of, some of the differences between these species and the traits they possess. What information could you gain about the relationships between these species from morphological studies? Do you think these species are closely related or not at all related? Could genetic investigations assist with providing further information?

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WA Museum: photographer Terry Houston

FIGURE 8.1 The diversity of life in WA: **a** Mitchell's diurnal cockroach; **b** peacock spider; **c** ground shield bug; **d** Dawson's burrowing bee

The word 'evolve' means to develop gradually. It is important to note that the development of an individual organism is not considered evolution. Individual organisms do not evolve. The changes in populations that are considered evolutionary are those that are inheritable via the genetic material that is passed on from one generation to the next. If there are enough changes in the gene pool of a population, a new species may arise. Evolution may be slight or substantial; it embraces everything from slight changes in the proportions of different forms of a gene within a population, such as the alleles that determine the different human blood types, to the alterations that occurred on the path from the earliest organisms to dinosaurs and humans.

A history of evolutionary thought

In the late 1600s, Western civilisations believed in the idea of 'natural theology' – that every kind of organism has essential, unalterable characteristics. As biological studies blossomed, naturalists began noticing variability in species, and the discovery of the remains of animals unlike anything seen before introduced the idea of extinction, which challenged natural theology: where did these giant animals come from, and where did they go?

Questions like this prompted naturalist Jean-Baptiste Lamarck to devise the **theory** of 'transmutation of species'. Lamarck suggested that organisms pass on to their offspring characteristics that they acquire during their lifetimes; that is, individual behaviours during the lifetimes of organisms were the mechanism that drives **adaptation**. Although now discredited, this was the first theory that embraced evolution.

The theory of evolution by natural selection

In the 1850s, two naturalists named Alfred Russel Wallace and Charles Darwin were studying and collecting forms of life in different parts of the world. Working separately, they both arrived at the same idea about how species 'came to be', refuting Lamarck's theory. Darwin referred to 'this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection'. Originally published in 1859 as *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*, Darwin's work is now commonly known as *The Origin of Species*.

Natural selection is the principle theoretical mechanism for evolution. The theory of evolutionary change through natural selection links all species to a **common ancestor**. An **ancestor** is a species from which other species have evolved, and a common ancestor refers to an ancestor that is shared by different species. This is supported by molecular evidence: the fact that there is a common genetic code in the form of DNA and RNA. A theory is an explanation that has not been proven as fact but is supported by evidence. The evidence for the theory of evolution, discussed in this chapter, is a substantial body of observed data collected over many years.

Diversity in nature
An evolutionary biologist and his photographer have captured images of 39 species of birds of paradise. Birds of paradise are renowned for their beauty, and for their peculiar and outrageous adaptations.

The basis of Darwin's theory of evolution was that individuals within a population showed a range of variation in their characteristics. Those with characteristics, or traits, most suited to their environment would have an advantage over other individuals, making them more likely to survive and pass these traits to the next generation. In each generation, the favourable traits (i.e. alleles) would become more common, and the population would gradually change to become better suited to its environment. Darwin provided evidence for descent with modification (branching evolution) in the form of patterns in variation of domesticated and wild species, and patterns in species' distributions in time and space.

This was a new approach to understanding evolutionary relationships. Much of the previous work had viewed relationships between organisms to be like a ladder, and assumed that life could be organised into a hierarchy of lower to higher organisms. Darwin proposed a 'tree-like' scenario, in which life's lineages could be mapped on a branching diagram. Using this analogy, the forks in

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the branches mark points at which new species arise – evolutionary events that occurred when populations became so different from other populations of the same species that they could no longer interbreed. This important concept is the basis of **phylogeny**. Phylogeny seeks to reconstruct the evolutionary history of any given group of organisms, studying the similarities and differences between them. Evolutionary biologists are still wondering, though, how life first started. How did the inanimate transform into the animate?

Key concept

Evolution is gradual: organisms with small and favourable genetic changes survive more often due to natural selection; these changes are passed to the next generation and accumulate over a long time.

The detailed history of the evolution of today's many life forms is complex, and our path in coming to understand those details has involved a great many hypotheses, investigations and analyses. The contemporary view of evolution, the modern evolutionary synthesis, has come from more than 150 years of research and observation. There are currently five main sources of evidence for the theory of evolution: biogeography (long term studies on life on earth) comparative genomics (genetics), the fossil record (palaeontology), comparative embryology (developmental biology) and comparative anatomy.

Question set 8.1

REMEMBERING

1 Define
   - **a** evolution
   - **b** common ancestor.

2 For how long has life has existed on Earth?

UNDERSTANDING

3 Explain the similarities and differences between Lamarck's theory of transmutation of species by spontaneous generation and Darwin's theory of evolution by natural selection.

4 Compare the definition of evolution with the theory of evolution.

5 Explain why evolution is applied to a population, rather than an organism.

8.2 LIFE HAS CHANGED AND DIVERSIFIED OVER TIME

The life and times of Earth (so far)

Scientists use a geological timescale within which they can place the major changes that have occurred in the history of Earth and of life. Time has been divided into segments covering millions, sometimes billions, of years. First, geologic time is divided into **eons**, then eons are split into **eras**, eras into **periods** and periods into **epochs**. Geologic time is usually expressed as **mya** (millions of years ago). Conditions on Earth have changed significantly over time, and populations have adapted or become extinct. One of the major changes on Earth is the process of **continental drift**, which includes the changes in the landmasses on Earth from one supercontinent called Pangaea to the number of continents we have today, and their relative movements. Key events that have occurred so far in Earth's time line are summarised in Table 8.1.

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TABLE 8.1 Geologic timeline and key events in the history of life on Earth

EONS	ERAS	PERIODS (AND EPOCHS)	MYA	CONTINENTAL ASSOCIATIONS	LIFE
Hadean, Archaean and Proterozoic	Precambrian		4560–542		First life (prokaryotes: Archaea) 3.5 mya First bacteria First eukaryotes First multicellular organisms
Phanerozoic	Palaeozoic	Cambrian	542–488	Australia is part of Gondwana	First invertebrates Arthropods (including trilobites) Diverse marine communities Jawless fish First land plants and arthropods Jawed fish First trees First land vertebrates
		Ordovician	488–444		Ferns dominant
		Silurian	444–416		Swampy forests Vascular plants diversify
		Devonian	416–359		Insects appear – the first winged animals – and become dominant
		Carboniferous	359–299	Gondwana moves south	First reptiles and amphibians – become dominant
		Permian	299–251	Laurasia and Gondwana unite to form Pangaea	Reptiles dominant, rise of reptilian ancestors of mammals
	Mesozoic	Triassic	251–200		Catastrophic mass extinction eliminates most life. Surviving organisms start to diversify, including dinosaurs First mammals Dinosaurs dominant
		Jurassic	200–146	180 mya: Pangaea begins to break up 160 mya: Africa breaks from Gondwana	Gymnosperms are the dominant plants

Super fossil finder
Follow the instructions to simulate a fossil dig. Align the fossils with their geological time period.

→

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EONS	ERAS	PERIODS (AND EPOCHS)	MYA	CONTINENTAL ASSOCIATIONS	LIFE
		Cretaceous	146–66	Remaining part of Gondwana breaks up	First flowering plants (angiosperms) Arrival of marsupials in Australia via Antarctica Dinosaurs populate huge rift valley between southern Australia and Antarctica
	Cainozoic	Palaeogene (Palaeocene, Eocene and Oligocene epochs)	66–23	Australia begins to break from Antarctica and drift north Inland seas form as eastern highlands lift Antarctic ice cap begins to form	Dinosaurs now extinct Flowering plants, birds and mammals radiate into newly vacant **niches** left by dinosaurs First *Eucalyptus* species Rainforests contract to the equator
		Neogene (Miocene and Pliocene epochs)	23–3	Separation of Australia and Antarctica complete	First *Acacia* species Large marsupials are well established
		Quaternary (Pleistocene and Recent/ Holocene epochs)	3–present		Australia close enough to Asia to allow exchange of plants and animals (e.g. bats and rodents) Major ice ages First humans arrive and increase in range

Species extinction due to changes in climate, sea level and atmospheric oxygen

Over the course of its history, Earth's climate has oscillated between hot, humid periods and cold, dry periods. Evidence for this has been found in ice cores drilled in Greenland and Antarctica. Some ice cores are several kilometres long and contain a record of the climate dating back 100 000 years. It appears that past fluctuations have been dramatic.

For much of its history, Earth was considerably warmer than it is today, and the temperature gradation from the equator to the poles was not as great. In contrast, towards the end of the Precambrian era, over a period including the Carboniferous and Permian periods, and from the Oligocene epoch till around 12 000 years ago, snow, glaciers and sheets of ice covered much of Earth. Between these cold, dry periods there were long periods, millions of years long, of warmer temperatures, when the ice melted, the sea level was higher, humidity was generally higher and vegetation was generally more tropical. Such climate variations inevitably affect life, and it is possible to track some of these changes through the **fossil** record, especially fossil plants.

A critical environmental factor for life is the composition of the atmosphere. Earth's first atmosphere most likely had little oxygen. Evidence from ice cores shows that the oxygen concentration began to increase about 2.5 billion years ago. By 1.5 billion years ago, the level was about 1% of the present level. By 600 mya it had risen to about 5% of the present level. The increase in the level of oxygen in the atmosphere is credited to photosynthetic cyanobacteria

living in the oceans. Cyanobacteria were able to use carbon dioxide and water to produce organic compounds and oxygen, much like plants do. It is likely that increased levels of oxygen in the atmosphere enabled the growth, evolution and diversification of eukaryotes, which relied on oxygen for respiration.

Biogeography

Biogeography is the study of the distribution of organisms and ecosystems across the world and through geologic time. The fauna and flora of Australia owe their uniqueness to the isolation of the landmass. However, Australia and other landmasses in the southern hemisphere share many plant and animal groups. By looking at the pattern of these distributions today, and that of the fossils, we are able to reconstruct its evolutionary history.

Many genera of Indian plants are similar to those of the monsoonal environments of northern Australia. Some Malaysian rainforest genera occur in the rainforests of tropical eastern Australia. Southern beech trees, *Nothofagus*, are found as both living and fossil specimens in mainland Australia, Tasmania, Papua New Guinea, New Caledonia, New Zealand, Antarctica and South America (Figure 8.2). The mountains and dry valleys of Antarctica have fossils of *Glossopteris* seed ferns (embedded in rocks and coal seams) that are the same as those found in coal deposits in India, South America, South Africa and Australia. The far-flung distribution of these groups provides evidence that these countries were once connected as Gondwana.

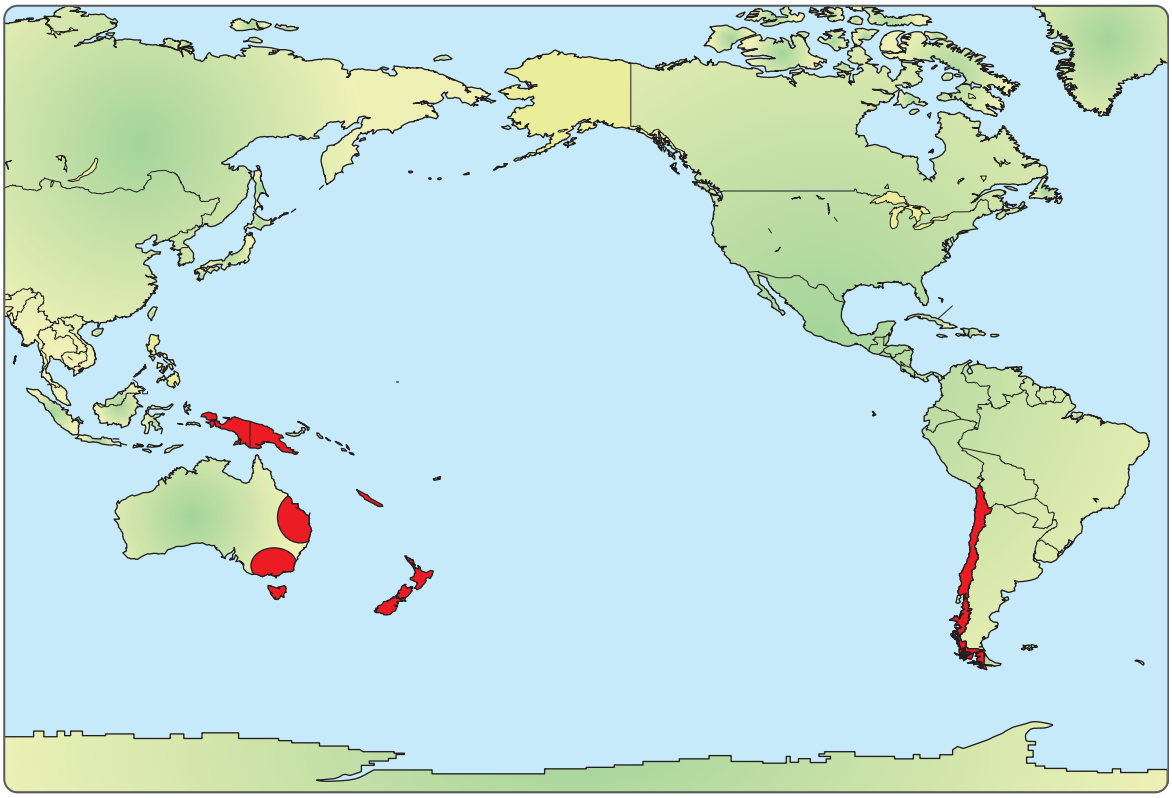


FIGURE 8.2 The red-shaded areas show where southern beech trees, *Nothofagus*, are found as both living and fossil specimens in Australia, Papua New Guinea, New Caledonia, New Zealand and South America.

Key concept

Evolution of new species arises gradually and has been influenced by geographical changes over time.

Question set 8.2

REMEMBERING

1 Define biogeography.

UNDERSTANDING

2 Describe how the study of biogeography can provide evidence for evolution.

3 Discuss possible effects on a population of large land herbivores if their climate becomes warmer and wetter.

4 Refer to Table 8.1 (pages 251–252) to complete the following tasks.

- **a** Identify the era in which life first appeared.
- **b** List all periods in which dinosaurs existed.
- **c** Determine whether *Eucalyptus* species would be found naturally in Africa. Explain your reasoning.

8.3 EVIDENCE FOR THE THEORY OF EVOLUTION: COMPARATIVE GENOMICS

Genomics is the study of the whole set of genes of a species and the interactions of the genes within a genome. The genomes of many species have been fully sequenced. Complete genomes are now available for humans, chimpanzees, koalas and bacteria, among others. Some organisms share **molecular homologies** with one another, as well as the observed structural (morphological) ones. **Homology** is similarity between a pair of structures, or genes in the case of molecular homology, due to shared ancestry. DNA itself is a very simple example of a molecular homology that links all life on Earth. Both DNA and RNA possess a four-base code that is shared by all living things. All organisms possess sequences of the same four nucleotides. This is known as the universal genetic code and is evidence for all life having a common ancestor.

By comparing the genomes of different species, sets of conserved genes that define a taxon's characteristics can be determined. A **taxon (plural taxa)** is a named group of organisms, such as beetles or reptiles. A **clade** is a group of organisms that includes all the descendants of a common ancestor and the ancestor species itself. For example, birds, dinosaurs, crocodiles and their common ancestor form a clade. Scientists use this information to measure the **relatedness** of two species. Relatedness is a measure of evolutionary distance. The relatedness of groups of organisms is reflected in the similarity of their DNA sequences. Taxa that share a more recent common ancestor with one another are more closely related than are taxa with a less recent common ancestor further in the past.

Comparative genomics

Comparative genomics is a field of biological research in which researchers use a variety of tools to compare the genome sequences of different species. The more similar in sequence the genes and genomes of two species are, the more closely related those species are in their evolutionary history, because less time has passed in which mutation and other genetic changes have accumulated. By determining the evolutionary relationships between species from the similarities and differences in their DNA, scientists can better understand how the appearance, behaviour and biology of living things have changed over time. Features shared by very different kinds of animals, such as humans and fish, can be encoded by identical genes sequences that have been **conserved** in both of them. Such sequences indicate that they share a common ancestor, even though, over time, they have diverged from one another.

On the other hand, when genomes of closely related species such as humans and chimpanzees have been studied, certain sequences have been shown to differ, and the nucleotide differences can be measured.

Comparative genomics
Read more about comparative genomics here.

Scientists don't always agree about the best way to measure genetic relatedness. Sometimes particular genes are compared. Sometimes repeated intron sequences (e.g. short tandem repeats) are compared. Sometimes all of the differences between the DNA sequences in the genome are compared. For example, the chimpanzee and human genomes have been compared, revealing a very close evolutionary relationship. The difference between their genomes is about 4%. The relatively few genes that differ in the genomes of closely related species are likely to be the genes that cause the observed differences between them and that make them unique. Additionally, by comparing the genomes of different species, scientists can gain information about the rate of change in genes. They have found certain genes have been changing (evolving) faster in humans than in chimpanzees.

In the past, **DNA–DNA hybridisation** methods have been widely used to analyse the relatedness of pairs of species, but they can be unreliable when comparing closely related species. In DNA–DNA hybridisation, DNA is extracted from two organisms, purified and cut into fragments. It is unwound and the hydrogen bonds joining the two sugar–phosphate backbones are broken. The resulting single strands of DNA from the two organisms are mixed. Some of the double-stranded DNA that forms contains DNA from each of the two species and is known as hybrid DNA. Some lengths of DNA will not pair up because the bases do not match (i.e. they are not complementary).

The double-stranded molecules are then heated. Greater similarity in the hybridised sequences means there will be more complementary bonds – the hybrid strands will bind together more strongly and be more resistant to separating when heated. The resistance to separating can be measured to work out evolutionary relatedness. The differences are detected as percentage hybridisation. More matching indicates the two organisms are more closely related.

Analysis of gene similarities has disproved some evolutionary trees that were based on structural (morphological) characteristics. For example, comprehensive analysis of the genes of wading birds, through both DNA–DNA hybridisation and DNA sequencing, has shown that the closest living relative of the flamingo is not a long-legged, graceful wading bird, as previously thought, but the squat grebe (Figure 8.3). The diving, piscivorous grebes have in the past been grouped with loons. Long-legged, long-necked flamingos have over the years been grouped variously with storks, waterfowl and stilts. Multiple DNA studies have confirmed, however, that flamingos and grebes are more closely related to each other than to the other groups of waterbirds.

Alamy Stock Photo/Brian Jannsen

iStock.com/purn_eva

FIGURE 8.3 Evolutionary relationships can be supported by DNA evidence, such as the genetic links between the **a** pink flamingo and the **b** horned grebe.

Through analysing variation in DNA or RNA sequences, scientists can obtain a measure of the difference between organisms and trace evolutionary relationships. Ribosomal RNA (rRNA) is

sometimes used in comparative genomics, because across different species the code is very similar and there are relatively fewer differences: it is a highly conserved code. Subtle phenotypic differences between species are due to mutations in genes and the resultant differences in the sequences of bases. Over evolutionary time, organisms very slowly accumulate changes in the sequences of the genes that encode the ribosome. Any large, rapid change is unlikely to persist, because the functioning of the ribosomes is so critical for all aspects of life and reproduction in an organism. The rRNA of two microbes that have been sequenced and compared is shown in Figure 8.4, illustrating the subtleness of the changes.

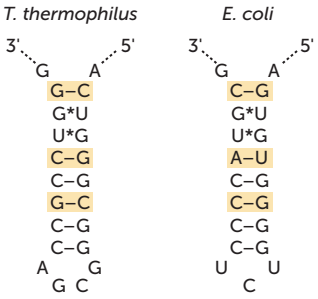


FIGURE 8.4 Small differences in rRNA base sequences between two microbes

Comparative genomics is made possible with bioinformatics

The comparison of genome sequences of different species or individuals gives a very broad picture of DNA sequence conservation and mutation frequencies, making it possible to trace evolutionary processes responsible for the divergence of two genomes. Comparative genomics, however, produces huge amounts of data that must be stored and analysed in a logical way.

The scale of the computational framework for this volume of biological analysis is huge. Only very recently has it become possible to undertake these analyses, thanks to **bioinformatics** – the culmination of advancements in engineering, computer science, mathematics and biology. Bioinformatics is the digital storage, retrieval, organisation and analysis of an enormous volume of biological data such as nucleotide and amino acid sequences from different species. Bioinformatics has dramatically increased the size, accuracy and scope of data sets, such as those needed for comparative genomics.

Bioinformatics has provided significant advances in our knowledge of the entire genomes of organisms, and this has revealed yet more evidence of evolution. The base-pair composition of genes of seemingly unrelated organisms that code for comparable structures is remarkably similar. For example, the protein that controls building eyes in vertebrates such as humans, which corresponds to the *PAX6 gene,* is more than 69% similar in its arrangement to the protein responsible for building the eyes of an octopus. The similarities in sequence, function and abundance of these genes across a broad spectrum of phyla are an example of homology, in this case at a molecular level. This is an example of how the identification of molecular homologies via comparative genomics can reveal the shared common ancestry of diverse species (Figure 8.5).

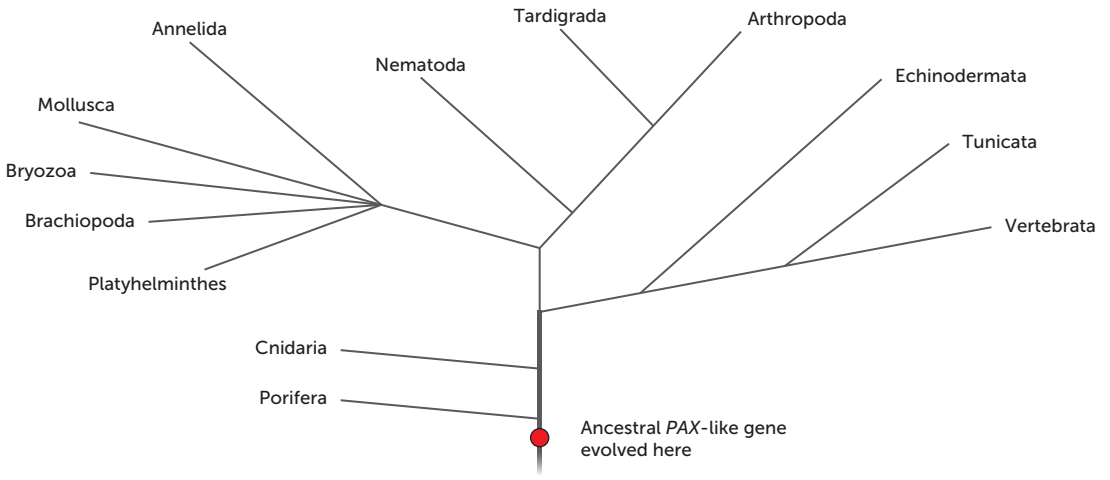


FIGURE 8.5 Comparative genomics has found shared eye-building genes across all animals with eyes. From these data, we can draw a phylogenetic tree.

Molecular homologies such as these also have application in building branching phylogenetic trees, a technique of **molecular phylogeny**. In the example of the eye-building *PAX6* gene, it is possible to conclude that as descendent lineages evolved, the gene was modified in a variety of ways in different lineages, giving rise to the diversity of eye-building genes seen in modern animals.

Phylogenetic trees

Evolutionary relationships between groups can be represented using phylogenetic trees. These diagrams show how organisms are related to each other, but the tree is hypothetical, not a certain fact. A phylogenetic tree can be built using physical information like body shape, bone structure, or behaviour, or it can be built from molecular information, like genetic sequences. Any DNA, RNA or protein sequence can be used to generate a phylogenetic tree; however, DNA sequences are most commonly used in generating trees today. The pattern of branching in a phylogenetic tree reflects how species or other groups evolved from a series of common ancestors. The more closely related species have a more recent common ancestor. The more distantly related species have a distant common ancestor.

TABLE 8.2 Phylogenetic tree vocabulary

TERM	DEFINITION
Species	A species (or sometimes another taxon, such as a group of similar species) is found at each tip of a phylogenetic tree. It is a group of organisms or populations that can only interbreed among themselves to produce viable, fertile offspring.
Tip	A tip is found at the end of a branch, where a species/taxon name is found. If the species/taxon is still living (not extinct), the name is aligned with the other species' names.
Node	Each point where two branches split is called a node. A node represents a common ancestor shared by at least two species. A node is the most recent common ancestor of all species on those branches.
Root	If you go all the way down to the bottom of the phylogenetic tree, the last node is called the root. This is the common ancestor of all the species in the tree.
Branch	A branch is a line drawn in the phylogenetic tree. At the end of the branch is the tip, where you find a species/taxon. The length of a branch can represent the divergence time.
Taxon	A taxon (plural taxa) is a named group of organisms, such as beetles or reptiles.
Clade	A clade is a group of organisms that includes all the descendants of a common ancestor and that ancestor.

Note: every species to the right of the root would have diverged from a single common ancestor at the root.

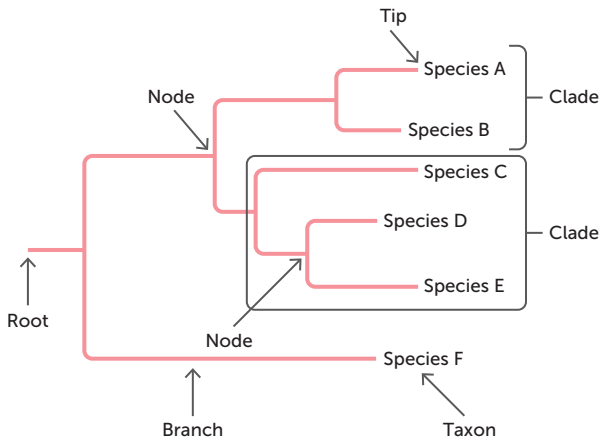


FIGURE 8.6 The relationships between parts of phylogenetic trees

Field guide: Squarish-corner tree
View the transition from one style to another; different styles can be used to show the same evolutionary relationships.

Differences between legless lizards and snakes include the facts that legless lizards lack venom glands, they cannot constrict prey and they have a fleshy tongue rather than a forked tongue. Morphological (physical) traits help us describe species, but they do not define them. Snakes and

legless lizards do have structural differences, but they also seem morphologically similar due to the absence of legs. However, DNA sequencing indicates that legless lizards and snakes evolved from different lineages of legged lizards. This is an example of **convergent evolution**, which is discussed later in this chapter.

SHUTTERSTOCK.COM/KEN GRIFFITHS

SHUTTERSTOCK.COM/ PHOTODIGITAAL.NL

FIGURE 8.7 a Legless lizard; **b** snake. Snakes diverged from their common ancestor a relatively long time before legless lizards.

To build your own phylogenetic tree, to show relative evolutionary relatedness (how recently two species shared a common ancestor), you need to know about either morphological differences or genetic differences. Use the guidelines in Worked example 8.1 to practise constructing your own phylogenetic tree.

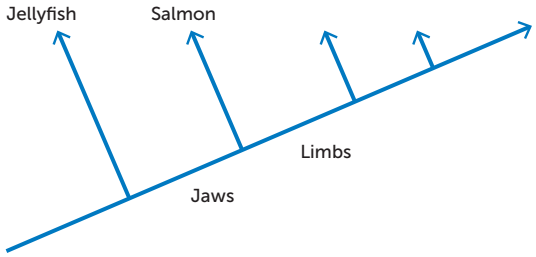
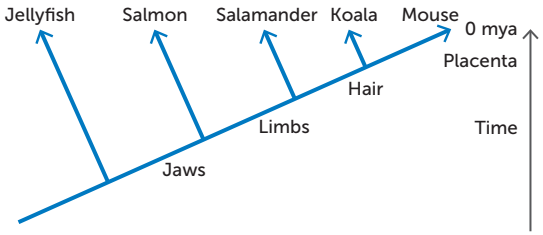
Worked example 8.1

Draw a phylogenetic tree

Consider the following table of data and use it to construct a phylogenetic tree.

ANIMAL	JAWS	LIMBS	HAIR	PLACENTA
Salamander	Yes	Yes	No	No
Mouse	Yes	Yes	Yes	Yes
Jellyfish	No	No	No	No
Koala	Yes	Yes	Yes	No
Salmon	Yes	No	No	No

STEP	VISUAL AID
1 After arranging your species in a table of differentiating features, find the species that is the most different from the other species. This is the species that diverged first. It is the one that does not have any of the traits observed and is called the outgroup, The simplest hypothesis is that it has the most distant common ancestor with all the other species and is the most distantly related to them.	 **FIGURE 8.8** The most distantly related species have the most distant common ancestor.
2 Draw a deep branching out point to show there is a further common ancestor not mentioned. Then draw your second branch to a tip to include that first distantly related species you identified in step 1.	

STEP	VISUAL AID
3 Find the feature that all of the remaining species have in common, and label the first section of the diverging branch with that feature. Next, find the species that only has that one feature in common with the others, and draw a branch from the main branch to a tip. Write the name of this species at the tip.	 **FIGURE 8.9** Draw the next branch to represent the species with one feature in common with the remaining species.
4 Find the next most common trait. Write the trait on the next section of the tree, showing that the remaining species have this in common. However, find the species that *only* has that in common, and draw another branch showing it diverging after this trait has appeared.	
5 Continue until all the species have diverged. Remember the tree is a set of hypotheses, and when you are unsure about which species to diverge next, opt for the simplest explanation (parsimony).	 **FIGURE 8.10** The tree is a set of hypotheses.
6 Draw an arrow alongside the tree to indicate the direction of time from the past to the present. Time is usually measured in mya, zero being at the tips of the branches.	

How to draw a phylogenetic tree
Watch the video and draw along with the host.

Mutation rate

In the absence of external influences, such as ionising radiation and chemical mutagens, a baseline rate of mutation occurs naturally in DNA. If mutations cause a change in the structure or function of the proteins that are encoded by the DNA, they may affect whether those proteins are passed to the next generation, and they will become either more or less common in subsequent generations. In many cases, mutations arise in non-coding regions, or may change a codon to one that encodes the same amino acid as before, resulting in a neutral mutation. The frequency of new mutations in a single gene or organism over time is fairly constant within a species and is called the **mutation rate**. When comparing the genomes of two species, the mutation rate can be used as a molecular clock to estimate at what point in time those species diverged from a common ancestor. For humans, the mutation rate is estimated to be approximately 10^{-8} (changed nucleotides) per nucleotide base pair per generation.

Comparative biochemistry and protein conservation

Organisms consist primarily of organic compounds, including proteins. They rely on enzymes to control chemical reactions, and they share a similar cell membrane structure. The amino acid sequence of certain proteins found in many organisms (such as haemoglobin and cytochrome-c) has been analysed across a range of organisms, and the similarities provide evidence for evolution. **Comparative biochemistry** is the study of different kinds of proteins (including enzymes), their fundamental units (amino acids) and cell machinery. It involves analysis of the similarities and differences, and the results enable evolutionary biologists to estimate relatedness between species.

Proteins, and the alleles that encode them, are subject to the same mechanisms of evolution as the larger traits that individuals possess. A protein that is well suited to its function will be preserved,

or conserved, while other traits around it may evolve. Two distantly related species may share very similar protein sequences for proteins whose function is much the same in those species, such as the histone proteins.

Mutations that arise over time may alter a protein's function, usually making it less suited to its function. If a point mutation results in the loss of an amino acid that is essential for the protein's function, the mutation may not be preserved. Protein sequences can be compared across species, and conserved amino acids can be identified. This is another line of enquiry when working out the evolutionary relationships between different species.

Occasionally, mutations may arise that change an encoded amino acid to one with a very similar charge and shape. Thus, the protein is still essentially conserved, as the substituted amino acid will allow the protein to have the same function.

Proteins consist of long chains of amino acids, and each protein differs in the number, type and sequence of its amino acids. The number of amino acid differences in the same protein in different species is used to determine the relationship between species. A small number of differences indicates a recent divergence from a common ancestor. A large number of differences indicates a more distant evolutionary relationship. For example, the differences in sequence of the 146 amino acids that make up the blood protein haemoglobin are an indicator of the closeness of the relationships between certain primates, as shown in Table 8.3. These comparisons indicate that chimps and gorillas are the nearest living relatives to humans.

TABLE 8.3 Differences in the amino acid sequence of haemoglobin between humans and other primates

PRIMATE	NUMBER OF DIFFERENCES IN THE AMINO ACID SEQUENCE
Chimpanzee	0
Gorilla	1
Gibbon	3
Orangutan	4
Macaque (monkey)	8
Lemur	5

Key concept

Comparative genomics utilises biotechnology to study the genome of a species and to compare the genomes of different species. Relationships between species can be displayed using phylogenetic trees.

Question set 8.3

REMEMBERING

1 State the purpose of a phylogenetic tree.
2 Define bioinformatics.

UNDERSTANDING

3 Define:
   a comparative genomics
   b comparative biochemistry.
4 Explain the advantage of mass data storage (provided by technological advances in the use of bioinformatics) when determining the relationships between seemingly unrelated taxa.

5 Legless lizards and snakes are both legless; however, legless lizards are more closely related to other species of lizards than they are to snakes. Use the image below to answer the following questions.

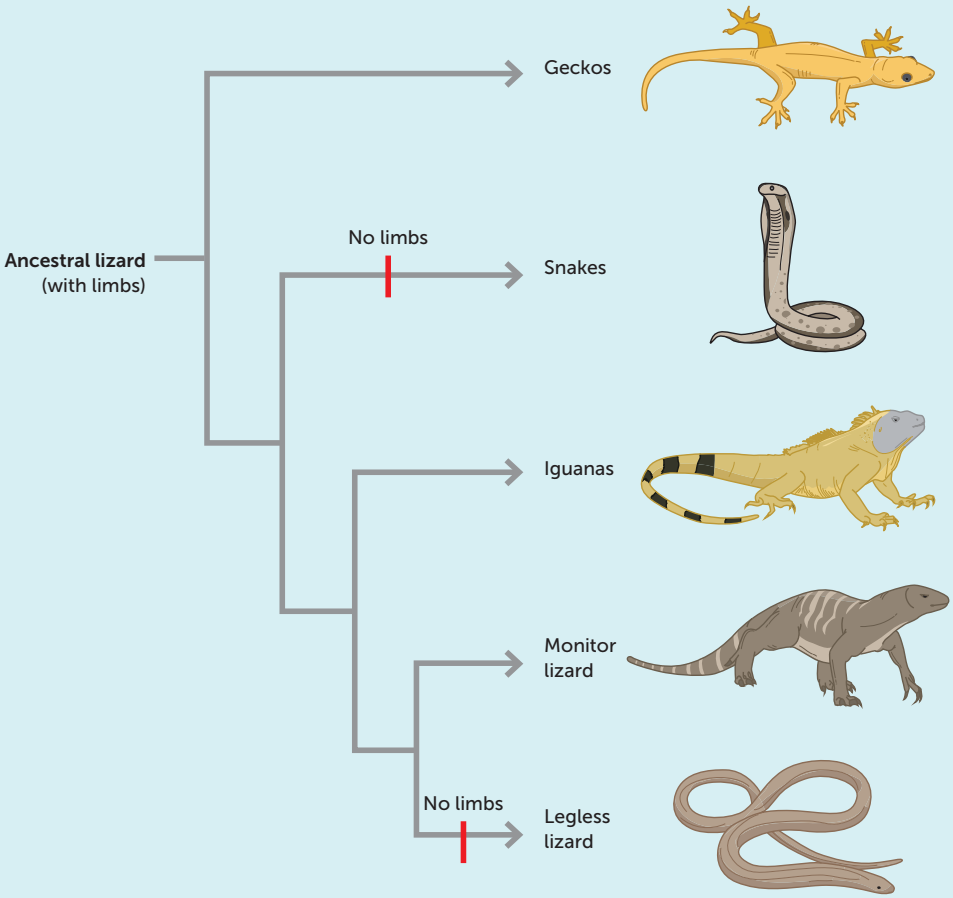


FIGURE 8.11 Reptile phylogenetic tree

a Name the animal that is most closely related to the legless lizard.
b Which animals are in the same clade as geckos?
c Draw the same phylogenetic tree, representing the same evolutionary relationships, using a different style of tree.

CREATING

6 Construct a phylogenetic tree for the plant species found in the table below. You do not have to use all of the features listed.

	VASCULAR TISSUE (XYLEM AND PHLOEM)	SEEDS	CONES	SPORES	TRUE ROOTS	FLOWERS AND FRUITS
Bryophyta (e.g. mosses)	–	–	–	Yes	–	–
Filicinophyta (e.g. ferns)	Yes	–	–	Yes	Yes	–
Coniferophyta (e.g. pine trees)	Yes	Yes	Yes	–	Yes	–
Angiospermophyta (e.g. roses)	Yes	Yes	–	–	Yes	Yes

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8.4 EVIDENCE FOR THE THEORY OF EVOLUTION: THE FOSSIL RECORD

Fossils are the preserved remains and traces that provide evidence of past life. These remains can be hard parts, such as teeth, bones and shells, or impressions in the rock where the organism's tissue has decayed. Most fossilised hard parts of animals or plants are found in rocks that have been derived from sediment, that is, sand, silt or clay. Along with animal bones, such as the skeleton of *Ceratoichthys* (Figure 8.12), fossils can also include footprints, burrows and even preserved waste products such as coprolites (fossilised faeces). The study of fossils is called **palaeontology**.

What can the fossil record tell us? Much of our knowledge of the changes that have occurred in living things over time is derived from fossils. Only a very small percentage of organisms leave fossilised remains. Many fossils are destroyed by natural processes such as weathering and erosion. Even so, fossils show that there has been a clear change over time from simple to very complex organisms, which is evidence for evolution.

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FIGURE 8.12 An immaculately preserved fossil of the extinct fish *Ceratoichthys*: a rare example of a complete fossilised skeleton

Fossilisation

The process of fossilisation requires very specific, and rare, conditions. The remains of the vast majority of long-extinct animals may never be found because they have been destroyed. Consequently, the fossil record is incomplete and biased toward organisms that lend themselves more easily to fossilisation. To become a fossil, organic matter needs to quickly be deposited and covered in sediments, creating an environment that lacks oxygen, preventing decomposition. Plant and animal remains can be preserved if they are covered in waterborne mud, sand or clay, depriving the remains of oxygen, as can happen in the beds of lakes and rivers or in calcium-rich sea beds. In many fossils, minerals from the sediments have replaced the natural bone or shell material, making the remains harder and more likely to fossilise. This type of fossilisation is called mineralisation.

Fossils can form when organisms are covered with sedimentary material, such as mud, silt or sand, generally carried by rivers and streams and deposited. These materials are consolidated to form sedimentary rock. This overlying sedimentary material protects organic matter from scavengers and also slows its decay long enough for it to fossilise. The resulting fossils generally only contain the hard parts of organisms (which are slow to decay), but on rare occasions they can include more delicate tissue such as feathers. Fossils of this type are not found in volcanic rocks, because molten lava solidifies at about 1000°C, which is hot enough to burn any organic material; however, they can be found in sedimentary layers of eroded volcanic ash. Metamorphic rock does not usually bear fossils, because the pressure and heat of metamorphism generally (although not always) destroys any trace of fossils.

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Thin tissue, such as leaves and muscle, is sometimes preserved as films or impressions left in the rock. Fossils are also formed when soft material, such as volcanic ash, fills an impression, or when minerals later form in a pocket in sedimentary rock left by a decomposed organism, which can result in fossils composed of opal. A 3.8-million-year-old set of footprints from a family of early humans, including children, has been preserved in this way in the Afar Triangle region of Africa. Dinosaur footprints can also be found in sandstone and mudstone.

There are several other ways a fossil can form. It can form as a result of freezing and subsequent dehydration. Plants are also quite commonly fossilised. The original plant material may be partly dissolved and some tissue replaced with dissolved salts, which petrify the material (i.e. replace it with rock). Entire tree trunks have been preserved by petrification in fossilised forests in Arizona and Antarctica. Fossilisation can tell us a great deal about past life and how it differs from what we see in the world today. But in order for this to make any sense, we need to calculate the age of the fossils. This can be done using dating techniques.

Fossil 'platypus' jaw found
Read about an ancient Australian monotreme

Ancient platypus found in New South Wales

8.1 APPLICATION

The jaw bone of an ancient relative of the platypus was discovered at Lightning Ridge in New South Wales. At more than 100 million years old, this is one of Australia's most ancient mammal fossils. It is a small jaw with three teeth beautifully preserved in translucent opal. The tiny details of the root and nerve canals can all be seen.

Principle of superposition

Fossils found in rocks lower down in the earth are older than fossils found closer to the surface (unless folding has occurred). The **principle of superposition** is fundamental to the interpretation of Earth's history, because at any one location it indicates the relative ages of the rock layers and the fossils within them. The basic principle is that the oldest rock layer is found at the bottom of the rock, with each consecutive layer above being relatively younger.

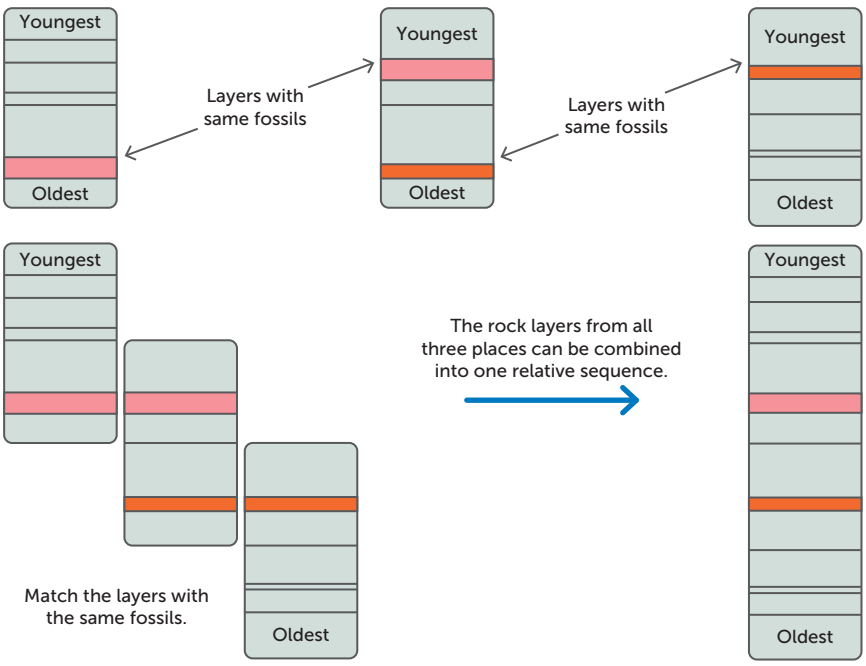

FIGURE 8.13 Studying fossils in rock layers at three different locations

The principle of superposition
Examine the cliffs and work out which layers were youngest and which were oldest.

Because fossils can be dated, the sequence of changes from the very earliest life to the present can be observed. The layers of rock in an area being surveyed form a profile. Each layer of rock in a profile is known as a **stratum (plural strata)**. The type of rock is often sedimentary but can be volcanic in origin. Volcanic ash or volcanic rock that has been eroded is sometimes compacted to form a special type of sedimentary rock that can be dated using radiometric dating. Strata are arranged in the order in which they were deposited, with the oldest layers being at the bottom unless they have shifted due to geological processes. Knowing the date of one layer can help position a strata in geologic history. Dating fossils according to the strata in which they are found is a relative dating method. It only enables palaeontologists to determine whether one fossil is older or younger than another fossil in a different stratum. **Absolute dating** tells the actual age of a fossil. Nearly all absolute dating methods utilise radioactive elements that occur naturally in the minerals or organic matter found in the fossil. Dating is discussed in more detail on page 266.

Transitional forms and the pace of evolution

There are many examples of intermediate states between an organism and its ancestral form, such as the famous dinosaur–bird *Archaeopteryx* (Figures 8.14 and 8.15). *Archaeopteryx* was a small flying dinosaur with feathers. It appeared in the late Jurassic period. It had features in common with both birds and reptiles, suggesting that birds evolved from reptiles. Its reptile-like features include a long tail, claws, no keel, solid bones, and teeth. Its bird-like features include a wish-bone, feathers and reduced fingers. Intermediate states such as *Archaeopteryx* are called transitional forms. Transitional forms exhibit common traits found in both the ancestral form and the more modern species. They give us evidence for evolution of major groups, documenting change over time on a broad scale. Transitions between species are harder to identify due to the limited nature of the fossil record.

FIGURE 8.14 A reconstructed model of the bird-like dinosaur *Archaeopteryx*, an example of a transitional form between unfeathered dinosaurs and modern birds

FIGURE 8.15 Cast of *Archaeopteryx lithographica*. The presence of feathers can be seen clearly in the fossilised specimen.

The evolution of the horse is a good example of how the fossil record can be used as evidence for evolution. Fossils reveal how changes in ancestral species led to the modern horse. Our understanding of the evolution of horse feet is derived from a scattered sampling of horse fossils from within the multibranched horse evolutionary tree. These fossil organisms represent branches on the tree and not a direct line of descent leading to modern horses. However, Figure 8.16 clearly shows the transitional stages whereby the four-toed foot of *Hyracotherium*, otherwise known as *Eohippus*,

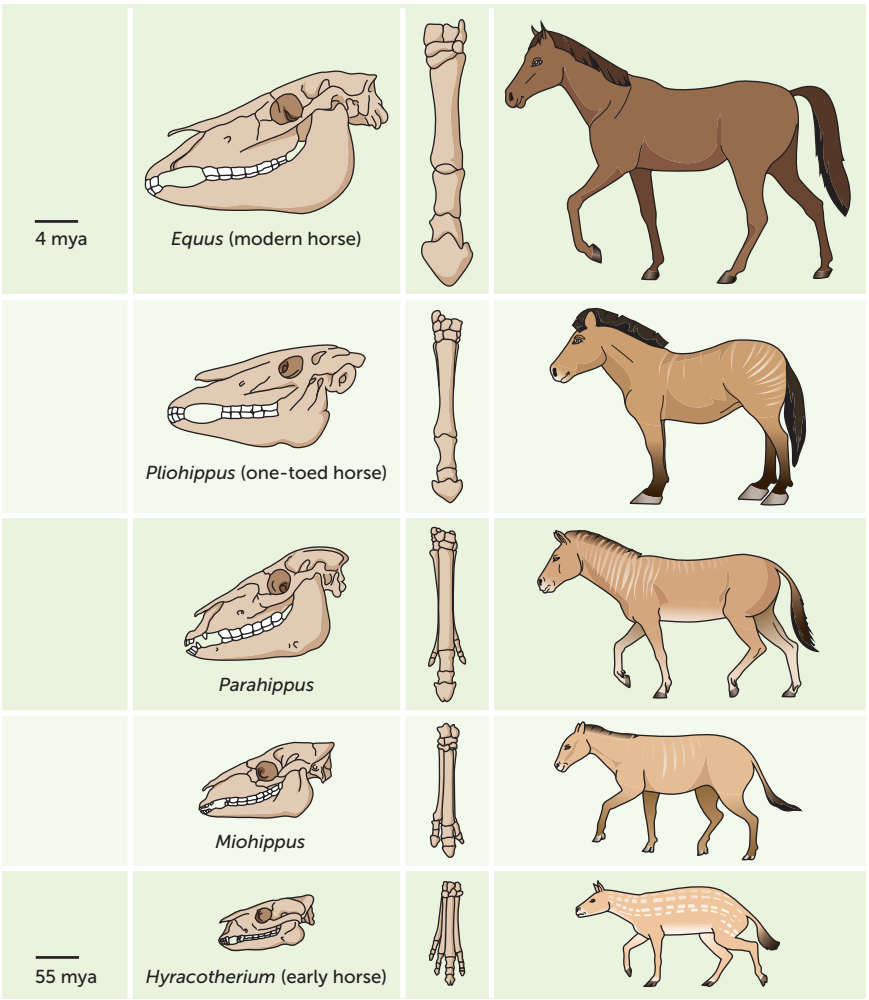

FIGURE 8.16 Transitional forms in the horse fossil record

gave rise to the single-toed foot of *Equus*. The transitional forms predicted by evolution were actually found to have existed.

Scientists have long hypothesised about the changes in horse species over time that are evident from the fossil record. The first horses were small, agile herbivores, well suited to the dense forest and soft terrain of their environment. Their multi-toed appendages distributed their body weight, preventing them from sinking too much into the soft ground, and their small size enabled them to duck and weave around closely positioned trees.

Horse anatomy and size changed as the environment changed. A cooler, dryer climate resulted in fewer trees and more grasslands, and the ground became harder. Anatomical changes in emerging species of horses included a reduced number of 'toes', which allowed them to manoeuvre efficiently and escape predators (to whom they were now more visible) and 'cheek-teeth' (molars), which enabled them to chew the tough grass that had become their food. Evolution continued, partly due to the mechanism of natural selection. Larger horses with better-developed molars had a survival advantage. The selective pressures of the environment meant that those horses would have been more likely to survive and pass their well-developed molars and fewer, shorter toes on to offspring. Traits that give individuals in a population a better chance of surviving selective pressures make them fitter than other individuals, so they are more likely to pass their alleles to the next generation.

Equus, a genus including zebras, donkeys, modern domestic horses and relatively recent fossil horses, has a taller body, longer legs and longer, squarer teeth than earlier horses. The changes depicted in Figure 8.16 occurred over an extended period of time – around 55 million years.

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There is always bias in the fossil record. Given the specific requirements for fossilisation and thus the limited nature of the remains that can be fossilised, there will always be chapters missing from the story. Even with this bias in the fossil record, it is possible to observe many examples of gradual change over time in organisms as their shape or size transitioned from one form to another. In other cases, no such gradual transition is evident – the changes seem sudden and inexplicable and the fossil record gives the impression of a burst of evolutionary speed because of what appears to be a gap. Such apparent gaps may be explained by aspects of two theories about evolutionary patterns: **gradualism** and **punctuated equilibrium**.

Gradualism

The concept of gradualism assumes that evolution occurs as a steady, slow divergence of lineages (ancestral tree branches) at an even pace. Gradualism states that apparently sudden bursts of evolution implied by the fossil record are not a real indication of an evolutionary history, but an illusion of the fossil record. Evolution only appears as a burst because of the absence of sediments containing fossils that document such a transition, or perhaps a change in conditions that made fossilisation impossible. Even if a small section of potentially fossil-bearing sediments were absent, this may account for fossils missing from millions of years in the fossil record. Were this section of strata still present, gradualism proposes, the fossils within it would show a divergence pattern that was slow, even and steady, in other words, gradual.

Punctuated equilibrium

In contrast to gradualism, the theory of punctuated equilibrium states that the apparent bursts of evolution are not an illusion, but real. It states that species remain fairly stable for long periods of time, but may swiftly change to a new species, for example, in response to rapid changes in the species' environment. Like gradualism, punctuated equilibrium accepts the existence of transitional forms between species, but over such brief periods that they were not preserved as fossils. Punctuated equilibrium proposes that there have been successive periods of stasis, each followed by a period of rapid change in a subset of the population.

Both gradualism and punctuated equilibrium are compatible with the theory of natural selection, and there appear to be examples of both in the fossil record. Whether there is relatively sudden or more gradual evolution could be expected to be related to whether change in the environment was sudden or gradual.

Key concept

Transitional forms exhibit common traits found in both the ancestral form and the more modern species. They give us evidence for evolution of major groups, documenting change over time on a broad scale.

Fossil dating methods

In order to make sense of the fossil record and to use it to learn about evolution, we need some basic information about fossils and their geological settings: how old particular fossils are, which organisms arose first, and which organisms lived at the same time. These questions can only be answered if we are able to accurately determine the age of the fossils. Both comparative and absolute dating techniques are used to estimate the ages of sedimentary rocks and the fossils within them.

The law of superposition
Read about the law of superposition and watch the animation that explains it.

Comparative dating

Comparative dating (also called relative dating) is used to determine the age of a rock, or a fossil contained in the rock, relative to other rocks or fossils found nearby. This approach to dating relies on our understanding of how sedimentary rock is formed (see page 262).

Sedimentary rock is composed of sediment: weathered material from the Earth's surface (such as gravels, silts, sands and muds) that have been transported by water and deposited in river beds, flood plains and sea floors. Sediment transport and deposition is an ongoing process; it has been continuously occurring on Earth for billions of years and can still be observed today. Over time, these deposited sediments form defined layers that consolidate into sequences of sedimentary rock. As discussed earlier, these sequenced layers are called strata, and a section showing successive layers of sedimentary deposition is called a stratification. Strata are deposited in a time sequence, with the oldest on the bottom and the youngest on the top, assuming natural processes like tectonic movement haven't twisted or inverted the layers. Palaeontologists assign relative ages to fossils based on the strata in which they are found. While this technique can't give an age in years, the sequence of the fossils can be deduced.

Absolute dating

Absolute dating refers to any technique that assigns a numerical age in years to a fossil or rock. There are three main types of absolute dating: **radiometric dating**, electron spin resonance and luminescence. Unlike comparative dating, which is based on assumptions about the sequence of strata (layers of rock), absolute dating is based on physical or chemical properties of materials in the rock.

Radiometric dating

The most common method of absolute dating is radiometric dating, which uses the known rates of decay of naturally occurring radioactive **isotopes** present in a rock or fossil. The various isotopes of an element have the same atomic number (the same number of protons) but a different atomic mass (different numbers of neutrons). For example, carbon has three natural isotopes: carbon-12, carbon-13 and carbon-14. Carbon-12 (^{12}C) has 6 protons and 6 neutrons in each nucleus, and carbon-14 (^{14}C) has 6 protons and 8 neutrons. Some isotopes have an unstable nucleus that emits energy in the form of radioactivity (alpha, beta or gamma rays) at a measurable rate. The half-life of an isotope is the time taken for half of the radioactive nuclei in an initial sample to decay.

Carbon-14 is a radioactive isotope that breaks down at a known rate to produce nitrogen-14 (^{14}N) (Figure 8.17). This measurable rate of decay is the basis of carbon dating.

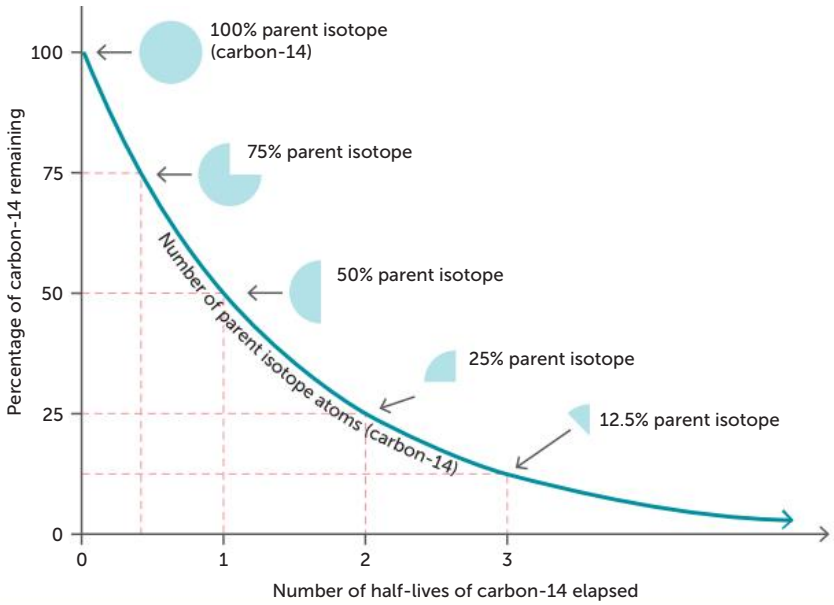

FIGURE 8.17 Graph of the half-life of carbon-14

Using the half-life of carbon-14 (5730 years), we can determine the age of the sample: in other words, the time taken for the original amount to decay to the present amount. The percentage of carbon-14 remaining compared with that of atmospheric carbon-14 can be converted into calendar

years. However, data from tree rings show that the amount of carbon-14 in the atmosphere can change with time. For this reason, the time calculated from carbon dating is usually expressed as $\pm x$ years.

The older the object, the greater the margin of error. Carbon dating is thought to be accurate for samples up to about 12 000 years old. After this time, it is difficult to measure the level of carbon-14 accurately; instead, other radioisotopes, such as potassium-40 (which decays into argon), are used (Table 8.4).

TABLE 8.4 Half-life and product of decay of some elements used in radiometric dating

ELEMENT	PRODUCT	HALF-LIFE (YEARS)
Thorium-232 (^{232}Th)	Lead-208 (^{208}Pb)	14 billion
Carbon-14 (^{14}C)	Nitrogen-14 (^{14}N)	5730
Rubidium-87 (^{87}Ru)	Strontium-87 (^{87}Sr)	48 billion

Carbon-14 dating is not always used on fossils for two main reasons: (i) in most cases fossils have been mineralised and the organic (carbon-containing) tissue has been chemically altered or replaced, and (ii) the process of fossilisation generally takes longer to occur than the maximum age of accuracy for carbon-14. However, by determining the various radioactive isotopes present in a sample containing a fossil, an age in years can be estimated for the sample and the fossil.

Key concept

While not complete, the fossil record provides evidence of evolution in transitional fossil forms. Applying the law of superposition (comparative dating) and using absolute dating techniques contribute to our understanding of the fossil record.

Question set 8.4

REMEMBERING

1. Recount the steps involved in the process of fossilisation.
2. State the principle of superposition.

UNDERSTANDING

3. Most of our knowledge of the evolution of sharks is based on the remains of fossilised shark teeth. Suggest why other fossilised body parts of sharks haven't been found in abundance.
4. Given we have more than just fossils available in the case of sharks, suggest alternative ways to determine how closely related various present-day animals may be to one another.
5. Palaeontologists have found tracing the evolution of sea jellies (jellyfish) to be very challenging. With your knowledge of fossils and the process of fossilisation, suggest why this may be the case.

APPLYING

6. A fossilised fish skeleton is found in sandstone, 1 metre below the surface, at location X. A very similar skeleton is found at location Y, 2 metres below the surface and 1 kilometre away from location X. A third similar skeleton is found at location Z, 3 metres below the surface and 3 kilometres away from location X. Describe what can be inferred about:
   a. the way in which the rocks were formed
   b. the age of the fossil at location Y.
7. Ancient stone tools have been found close to campfire charcoal. Explain how the technique of carbon dating could be used to determine the time at which the tools were made.

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8.5 EVIDENCE FOR THE THEORY OF EVOLUTION: COMPARATIVE EMBRYOLOGY AND ANATOMY

Close examination of the physical characteristics of species, at both the embryonic and adult stages, can reveal further evidence for evolution. **Comparative anatomy** is the study of the similarities and differences in structure between different organisms. Structural features are also called **morphological** features.

Embryology

Embryology is the study of the anatomy of embryos and how they develop over time until the adult stage. Comparative embryology is used to establish evolutionary relationships and common ancestry on the basis of the similarities and differences in anatomy and development between embryos of different species. It is thought that the longer embryos remain structurally similar during development, the more closely related they are. For example, all members of the phylum Chordata have, at some stage of their development, a dorsal notochord (a solid tissue running along the back), pharyngeal slits (which turn into gill slits in fish), a dorsal nerve cord and a tail that extends past the anus. The embryos of the different vertebrates are very similar and show features that are not present in adults. This suggests that these vertebrates evolved from a common aquatic ancestor, such as the crossopterygian fish (Figure 8.19, page 270).

Sea squirts are the most unlikely members of this phylum – the adults look more like marine invertebrates than the other vertebrates to which they are more closely related (Figure 8.18a). Sea squirt larvae, however, have the requisite characteristics for classification as being closely related to vertebrates, including a notochord (Figure 8.18b). Adult vertebrates have lost the notochord and it has been replaced with vertebrae.

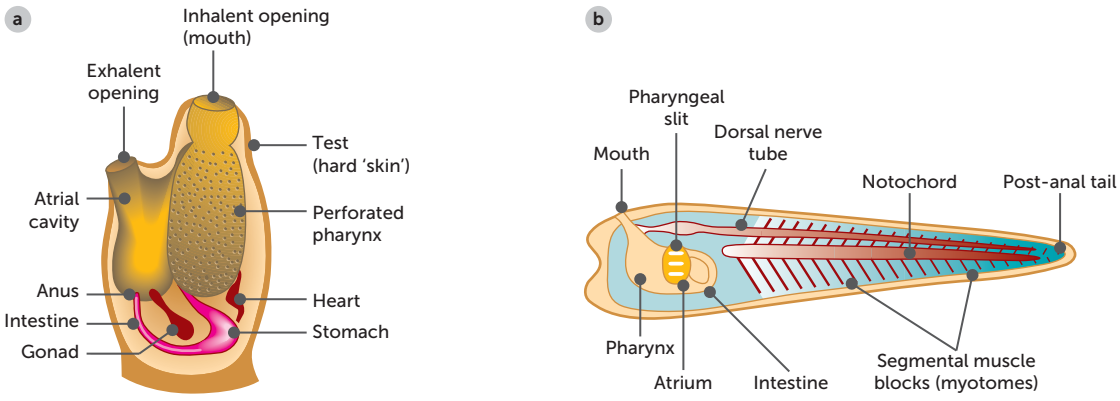

FIGURE 8.18 a The adult sea squirt shows few characteristics of chordates; **b** the free-swimming larva of the sea squirt, however, shows the characteristic features of chordates, revealing an evolutionary connection.

Key concept

Comparative embryology is the study of the similarities and differences between embryos of different taxa in order to establish evolutionary relationships.

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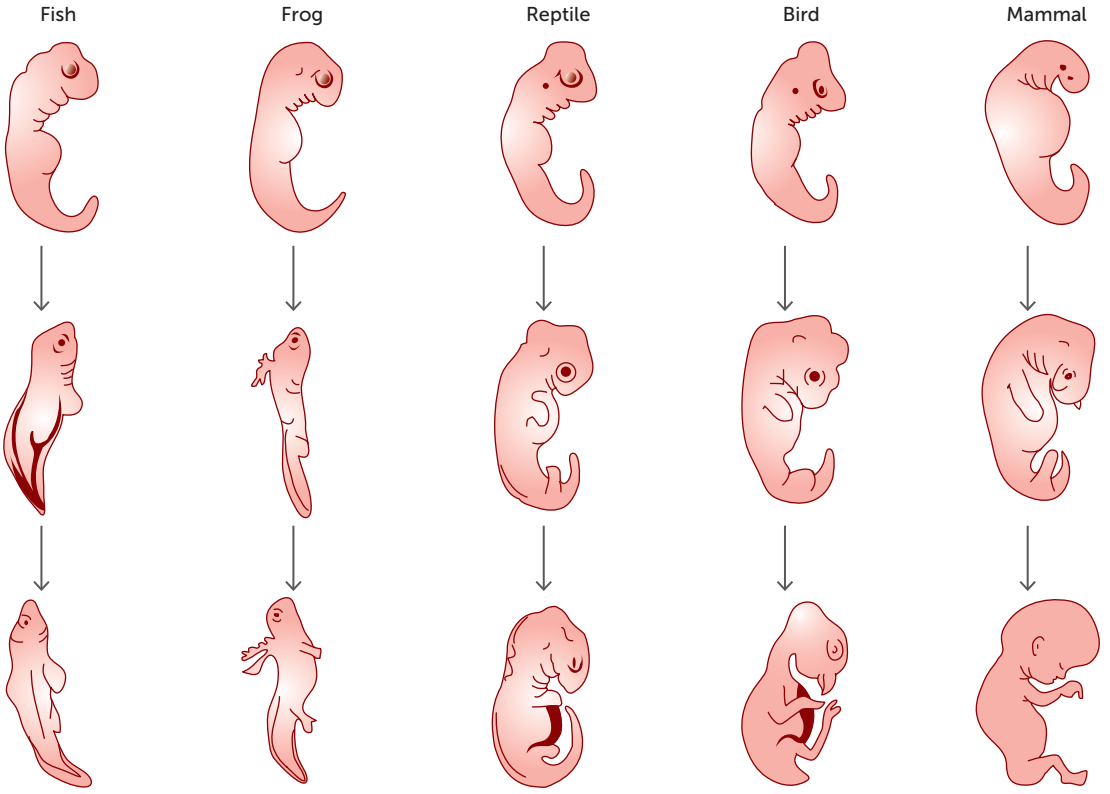

FIGURE 8.19 Similarities between chordate embryos suggest a common aquatic ancestor.

Comparative anatomy: homologous structures

The structures different species have in common can be evidence of inherited characteristics from a common ancestor. Comparative anatomy shows how seemingly disparate kinds of organisms actually share many fundamental similarities, and strongly supports the notion that those similarities are derived from a common ancestor. The differences we see in modern organisms are the result of changes over time as organisms have adapted to their various environments – in other words, evolution.

Anatomical structures that are common to more than one species and were inherited from a common ancestor, but have different functions, are known as **homologous structures**. Homologous structures show the same structural plan but perform different functions due to the different species living in different environments with different selective pressures (conditions).

Understanding evolution
Read more about comparative anatomy here.

When **adaptive radiation** occurs, organisms retain many of the same basic structures because they have the same genetic history. For example, all lizards have scaly skin; this is a defining characteristic of their classification. However, the scales can differ in colour, hardness and shape in response to the conditions in the habitat that they occupy, serving varying functions of defence, temperature maintenance or camouflage. The different types of scales are examples of homologous structures.

The lizard scale example of a homologous structure is one that has a relatively recent evolutionary history. Some homologous structures have evolved from a much more distant common ancestor, and they may have very different functions in the various taxa. The wing of a bird, the wing of a bat, the leg of a crocodile, the flipper of a whale and the arm of a human all have the same basic structure: the pentadactyl limb, with a hand or foot with five fingers or toes. In the different animal groups, however, it has been modified to suit a variety of different functions, demonstrated by the different bone lengths and coverings of the limbs (Figure 8.20).

The leaves of land and aquatic plants all have the same basic components, but they have enormous variety in size, shape, colour and function. Some leaves function as coloured petals, some as support

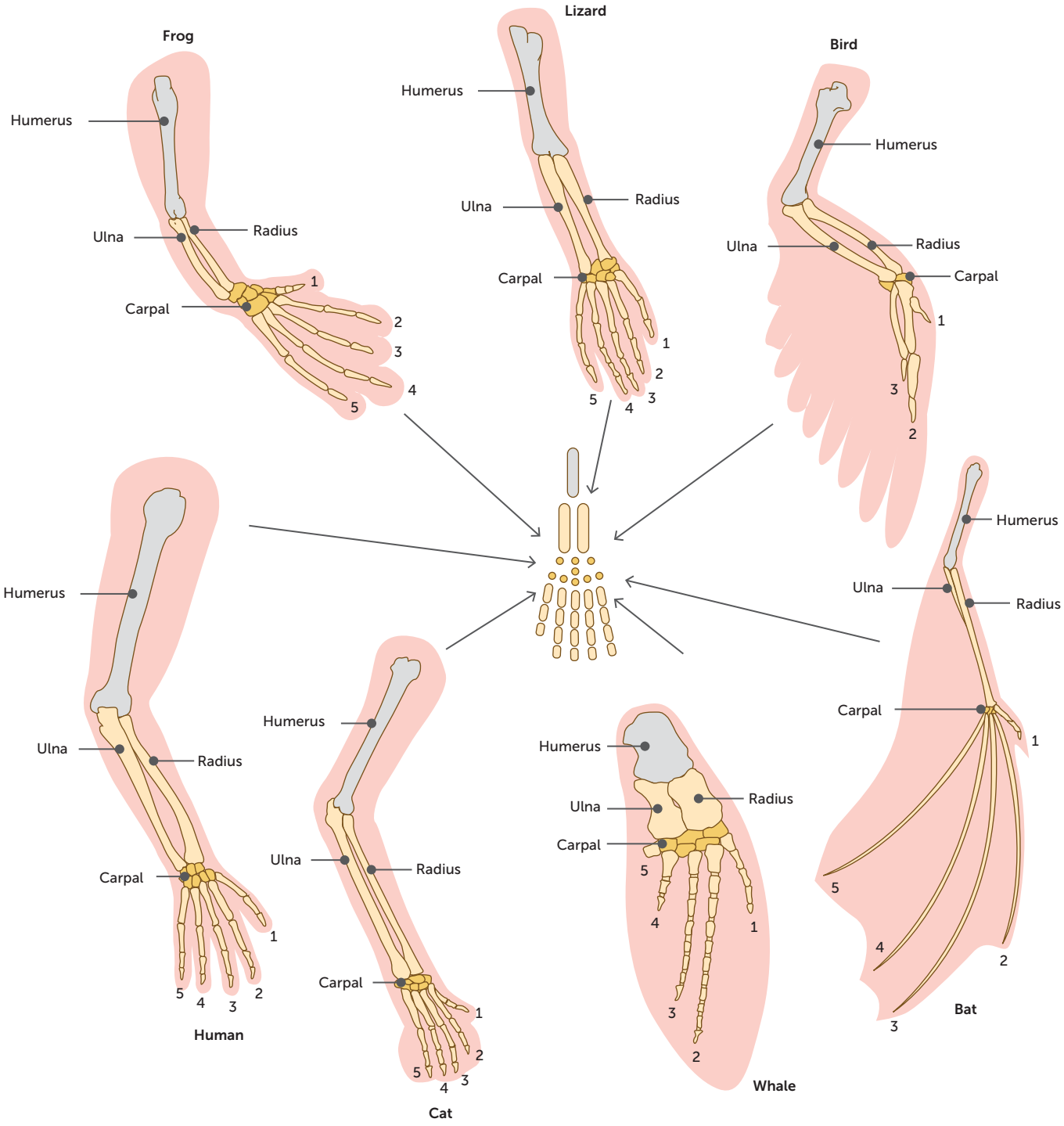

FIGURE 8.20 The principle of homologous structures is illustrated by the adaptive radiation of the forelimb of a selection of vertebrates. In each group it shows the basic pentadactyl pattern, but it has been modified for different uses.

structures in buds, and others as defensive spines or fleshy water stores. The plants in Figure 8.21 (page 272) demonstrate features that have been derived from the same basic structure, but now have different forms that serve different functions. In other examples, homologous structures can share the same function. The environment can influence the form and the use of homologous structures.

Homologous structures can be used to infer phylogenetic (i.e. evolutionary) relationships, because only organisms with a common ancestor are likely to have structures with the same basic arrangement.

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FIGURE 8.21 Homologous structures derived from leaves: **a** the spines of a cactus; and **b** the bracts of *Heliconia*

Vestigial homologous structures

In some cases, homologous structures stemming from a common descent can eventually cease to have any functional use for an organism; the structure may not necessarily impede a particular adaptation of an organism, but at the same time, it no longer serves a useful purpose. These structures are called **vestigial structures**. Vestigial structures are quite common and can take a variety of forms, including bones, soft tissues, organs, cells or molecules.

Vestigial organs are evidence for evolution, because it is hypothesised that they were once present and functional in their ancestors. Changes in the environment have rendered these organs redundant, so over time they have lost their functionality. They demonstrate the evolutionary divergence of a species from a past behaviour or activity.

An example of a vestigial organ is the pelvic bone in a whale – this bone serves no current purpose and is a remnant of a time when whales were terrestrial mammals.

Wherever vestigial structures may be found, they are usually either rudimentary or atrophied. Among humans, we need look no further than our vermiform appendix, a small, pouch-like structure on the colon. It appears to be the shrunken remains of the caecum, a far more extensive structure found in the digestive tract of other, more predominantly herbivorous primates.

Analogous structures

Analogous structures are features of organisms that have the same function but a different basic structures that evolved independently. The eyes of octopuses and vertebrates are remarkably similar, even down to fine points of detail, and an observer could conclude that they are homologous structures (Figure 8.22, page 274). However, in the vertebrate eye, the nerve fibres lie in front of the sensory cells of the retina, whereas in the octopus eye they lie behind them. Because of this, the vertebrate eye has a blind spot where the optic nerve emerges from it, whereas the octopus eye lacks a blind spot. The developmental process is different, which indicates that they are the products of two distinct lines of evolution. Bat and insect wings are another example of analogous structures.

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SCIENTIFIC LITERACY

'Terror bird' was a scary-looking vegetarian

Giant prehistoric 'terror birds' looked so fierce that many palaeontologists assumed they were terrifying predators, but new research finds that the supposed carnivores were probably herbivores.

The terror bird, aka *Gastornis*, grew to nearly 1.5 metres tall. It lived between 55 and 40 mya in what is now Europe and possessed a huge, sharp beak.

'The terror bird was thought to have used its huge beak to grab and break the neck of its prey, which is supported by biomechanical modelling of its bite force,' says Professor Thomas Tütken from the University of Bonn, who led the research.

'It lived after the dinosaurs became extinct and at a time when mammals were at an early stage of evolution and relatively small; thus, the terror bird was thought to have been a top predator at that time on land.'

Wrong, according to the latest findings, presented by Tütken and his team at the Goldschmidt conference in Florence this week.

An early clue came by way of footprints likely left behind by an American cousin of *Gastornis*. The footprints do not show imprints of sharp claws, which would have been expected as tools to grapple prey. Today's raptors, for example, sport such sharp claws.

Another clue is more obvious – the bird's hefty size and build. Can you imagine Sesame Street's Big Bird (with a big beak) running swiftly after prey? All of that bulk would not make for a very swift hunter. Some researchers theorised that terror birds ambushed prey, but even that seems pretty far-fetched.

To further explore the possibilities, Tütken and colleagues took a geochemical approach. They analysed the fossilised bones of the birds, focusing on calcium isotope composition. Isotopes are atoms of the same element with different numbers of neutrons.

In prior experiments, the scientists determined that the calcium isotopic composition becomes 'lighter' as it passes through the food chain. They tested the method first with herbivorous and carnivorous dinosaurs – including top predator *T. rex* – as well as mammals living today. For this latest study, they applied the method to terror bird bones housed at the Geiseltal collection at Martin Luther University in Halle.

They discovered that the calcium isotope compositions of terror bird bones are similar to those of herbivorous mammals and dinosaurs, and not to carnivorous ones.

'Tooth enamel preserves original geochemical signatures much better than bone, but since *Gastornis* didn't have any teeth, we've had to work with their bones to do our calcium isotope assay,' Tütken explains.

As for many scientific puzzles, the case isn't completely closed just yet.

'Because calcium is a major proportion of bone – around 40% by weight – its composition is unlikely to have been affected much by fossilisation,' he says.

'However, we want to be absolutely confident in our findings by analysing known herbivores and carnivores using fossilised bone from the same site and the same time period. This will give us an appropriate reference frame for the terror bird values.'

Even if the food was just plant based, it had to have been large and tough, given the impressive beaks the birds evolved.

Viegas, J. (2013) ''Terror bird' was scary-looking vegetarian', *Discovery News* online, 29 August 2013.

Questions

1. Suggest why it had been assumed that *Gastornis* was a predator, and what evidence may have pointed away from this before the so-called 'geochemical approach'.
2. Outline the reason for Tütken's caution in applying this geochemical analysis to fossil birds.
3. Suggest an alternative reason for the apparent absence of raptor-like toe claws on *Gastornis*.

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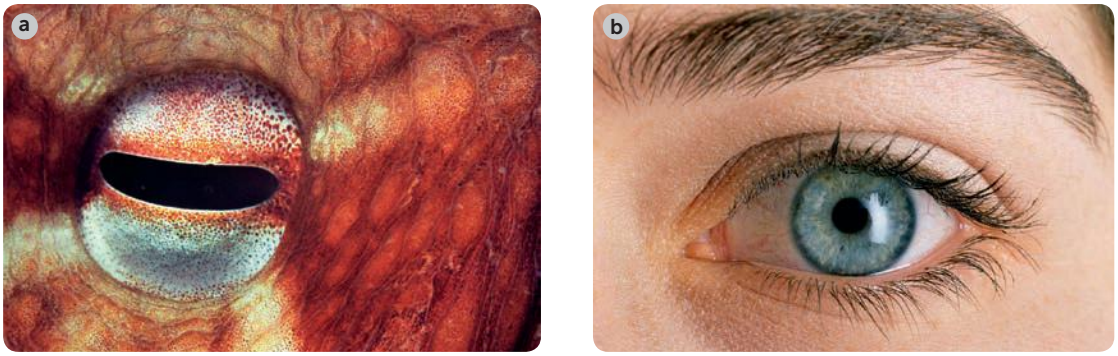

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FIGURE 8.22 a Octopus eyes and **b** human eyes are solutions to the same problem with similar adaptations, even though octopuses and humans are not closely related. An important control gene for building eyes, however, belonged to a very ancient common ancestor.

Key concept

Comparative anatomy involves comparing the morphological features of different species. Morphological features include homologous structures (same structure but different functions), vestigial homologous structures (same structure but no longer used) and analogous structures (same function but different structures).

CASE STUDY

Dr Erich Fitzgerald and the evolution of baleen whales

Dr Erich Fitzgerald is Senior Curator of Vertebrate Palaeontology at Museum Victoria. Erich researches the evolutionary history of marine mammals. To undertake his research, he uses a combination of field work and interpretation of the fossils and animal remains that are housed in museums around the world. Erich seeks to answer questions on what drives the evolution and extinction of marine mammals.

His research would not be possible without advances in information technology, such as computational phylogenetic analysis. 'To get to the bottom of the evolutionary history of organisms, you need to place them in an evolutionary context. To do that, we subject fossils and living species to phylogenetic analysis, computationally estimating the evolutionary "tree"' based on large data sets of characteristics across large numbers of taxa. We then use different programs to interrogate that tree for other patterns to test our hypothesis.

'Using computers as a way of capturing data, imagery, 3D and CT scanners and communication between researchers internationally has been a huge benefit to palaeontology; it means we are forced to access a wider range of data in order to get an accurate evolutionary picture of what we are looking at. Computers can deal with large data sets of measurements and characteristics and identify patterns that could easily be otherwise overlooked; as such, computers are as important to palaeontology as a hammer and chisel.'

Erich's research has unveiled unexpected results. 'For a long time, there was a gap in our knowledge of the relationships between the toothed ancestors of modern whales – Archaeocetes – and living baleen whales. How on earth does such a specialised feeding structure like baleen evolve? For some time, there was the idea that Archaeocetes probably closed their jaw and used their teeth like a sieve, like some seals do today.'

Extensive data sets, including measurements from the skulls, teeth and other bones of fossil and contemporary animals, allow for the use of computational phylogenetics, showing some surprising results. 'Our research points to a complex

story of "false starts" and "experiments" in evolution. It shows whales didn't have an intermediate stage using both teeth and baleen; the evidence suggests the transition between teeth and baleen happened a different way. The question is now how did this happen; that's what I'm trying to solve. Understanding the evolution of organisms is vital. In order to gain any understanding of the dynamics of biodiversity, you have to understand how it has occurred over the time scales over which it has evolved.'

Questions

1 Account for how our view of current biodiversity is biased if we ignore evolutionary history and the fossil record.
2 Using the example of baleen whales and their diet, assess and discuss whether the evolutionary 'false starts' and 'experiments' that the fossil record shows are examples of **divergent evolution**.
3 Prior to the advent of computer-assisted phylogenetic analysis, estimating evolutionary relationships of vertebrates was based largely on bone and tooth morphology, or shape. Phylogenetic analysis now incorporates other elements in order to develop phylogenetic trees. Explain how computational technology has made the identification of possible relationships of fossils and living animals more efficient and rigorous than was previously possible.

Newspix/Paul Trezise

FIGURE 8.23 Erich Fitzgerald and the fossil skull of the ancestral toothed whale *Janjucetus hunderi*

WA Museum
WA Museum was closed for four years until November 2020. It boasts a merger of culture and science. It promises to be somewhere to visit to see extinct species such as dinosaurs and newly discovered species.

Question set 8.5

REMEMBERING

1 Define morphological features.
2 Describe examples of homologous and analogous structures.
3 List two structural features found in the embryos of vertebrate animals that are not present in the adult form of the same species.

UNDERSTANDING

4 Compare homologous and analogous structures.
5 Describe how embryology provides evidence for the theory of evolution.

EVALUATING

6 Evaluate the reliability of vestigial structures as evidence for the theory of evolution.
7 Explain why seemingly unrelated organisms could have a high percentage of very similar genes.

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8.6 TYPES OF EVOLUTION: DIVERGENT VERSUS CONVERGENT

Divergent evolution

Divergence is a pattern of evolution in which differences between groups of organisms accumulate to a critical point that leads to **speciation**, the development of a new species. This pattern is usually the result of the dispersal of a single species to different environments; that is, groups from the same species become isolated from one another. The isolation stops the gene flow between these separated populations. The populations may have been separated by physical barriers such as mountains or rivers, or by other factors such as changes in reproductive timing.

A group of organisms that has a recent common ancestor may have evolved different adaptations in response to a range of environmental pressures. Homologous structures indicate that there has been divergent evolution, because new species have the same fundamental structural plan, but the structures may perform a different function.

Adaptive radiation

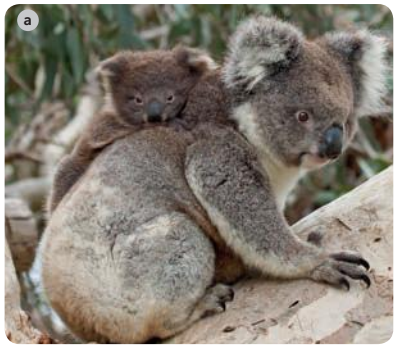
As members of the population develop adaptations, by natural selection favouring certain mutations over successive generations, they may diverge enough to become new species. This process is referred to as adaptive radiation.

For example, koalas (tree-dwelling herbivores), Tasmanian devils (ground-dwelling carnivores) and marsupial moles (dune-burrowing insectivores) are related because they have a common marsupial ancestor (Figure 8.24). However, they show quite different dentition (teeth) that enables them to consume different diets.

Koalas possess complex molar teeth (suited to chewing eucalyptus leaves) and blades on each tooth (which help cut the leaves).

Tasmanian devils have four pairs of upper incisors and three pairs of lower incisors that are long and sharp, suited to tearing meat and crushing bones.

The teeth of marsupial moles are unusual. They are degenerate and bear no resemblance to koala or Tasmanian devil teeth. The premolar is the largest of the anterior teeth. The incisors and canines vary in size, but are little more than conical projections, suitable for their insectivorous diet.



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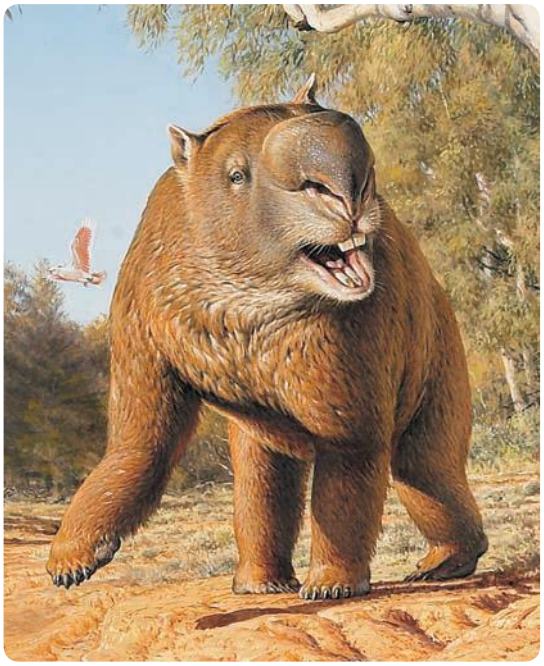
Science Source/Tom McHugh

FIGURE 8.24 Examples of the divergent evolution of marsupials: **a** koalas, **b** Tasmanian devils and **c** marsupial moles evolved from a common ancestor that probably lived during the Eocene epoch.

Adaptive radiation occurs particularly when environmental changes trigger the availability of new resources and environmental niches. A clear example of this can be found in Australia's fossil record, which indicates that during the Middle Miocene epoch (approximately 15 mya), dense tropical forests covered central Australia where the Simpson Desert is now. Forests, lakes and permanent rivers provided a lush habitat for marsupials, such as giant koala-like possums, shrewish insectivores and sheep-sized browsers. Flamingos, crocodiles, turtles and dolphins flourished in the waterways. The range of habitats allowed an extensive radiation of animal species, which adapted to the available resources and are, therefore, an example of adaptive radiation.

Slowly, the tropical centre of Australia began to dry out during the Pliocene epoch (approximately 5 mya). This brought an end to the tropical climate, which meant that the tropical forests gave way to broad grasslands. As the tropical forests retreated from central Australia, the animals they once supported were forced to compete for diminishing resources and became vulnerable to extinction. The large browsing mammals called diprotodontids (Figure 8.25) and a variety of possums were unable to survive the reduction in trees and the subsequent limited food availability.

The remnants of the tropical forests and their inhabitants are now confined to Papua New Guinea and pockets of northern Queensland. The grasslands that replaced the forests provided new habitats that allowed for adaptive radiation of other Australian mammals: the kangaroos and wallabies.



Artist: Peter Trusler © Australian Postal Corporation 2008

FIGURE 8.25 The giant *Diprotodon optatum* was a type of megafauna that browsed on leaves.

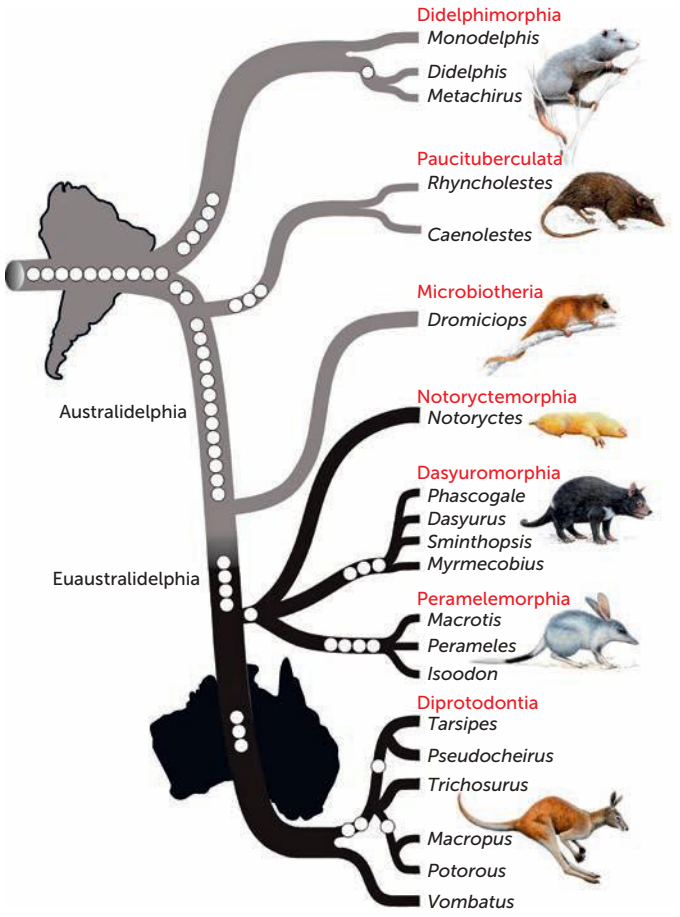


FIGURE 8.26 Adaptive radiation of marsupials began in South America, which was then joined to modern Australia in the supercontinent Gondwana. Most surviving marsupials are now restricted to the Australian continent.

Convergent evolution

Convergent evolution is a pattern that occurs when unrelated organisms evolve similar adaptations in response to their environment. An example of convergent evolution is provided by anteaters. Many animals eat ants and white ants (or termites), and they have developed similar structures, even though they are not closely related.

Modern anteaters include echidnas (which are monotremes), numbats (which are marsupials) and pangolins (which are placentals) (Figure 8.27). All of these species have an elongated snout that functions as a smelling and digging device, a long, extendible tongue that can extract ants from crevices, and powerful claws that are used for digging up ant and termite nests.

The different species of ant-eating mammals have a common ancestor, but not a recent one; they belong to different orders. They have developed ant-eating adaptations independently and coincidentally, rather than it being a legacy from their common ancestor. The first mammal-like animal probably emerged in the Triassic period, around 208 mya.

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FIGURE 8.27 Ant-eating mammals show convergent evolution in their ant-eating structures: **a** echidnas (monotremes); **b** numbats (marsupials); **c** pangolins (placentals).

The results of convergent evolution often show up as analogous structures: adaptations of very different types of structures that solve a problem in a similar way. The structures are genetically relatively different, but their functionality is very similar. Dolphins and sharks demonstrate convergent evolution. The dolphin is a mammal and the shark is a fish. They both inhabit the marine environment, which imposes the same selection pressures on both types of organism. Both groups have a streamlined body shape, and fins for propulsion and stability. These features are adaptive for movement in water. Analogous structures, such as the streamlined body, occur due to environmental pressures, not because they share a recent common ancestor. Instead, they share a very distant common ancestor.

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Key concept

Divergent evolution is when populations of a species differentiate to become separate species (e.g. different marsupial species). Adaptive radiation is an example of divergent evolution, and homologous structures provide evidence for this.

Convergent evolution occurs when species that are not closely related independently develop similar adaptations to their environment (e.g dolphins and sharks). Analogous structures provide evidence for convergent evolution.

Question set 8.6

UNDERSTANDING

1. Define divergent evolution and give an example of evidence for it in the evolution of species.
2. Define convergent evolution and give an example of evidence for it in the evolution of species.

ANALYSING

3. Classify the pentadactyl limb as homologous or analogous and give a reason why.
4. Explain the importance of using both living and fossil forms in constructing phylogenies.

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CHAPTER 8 ACTIVITY AND INVESTIGATIONS

8.1 ACTIVITY

Looking at fossils

Evidence of evolution comes from studying the organisms living today, but further evidence can be obtained by studying the animals and plants of the past as seen in the fossil record. Fossilisation is a rare occurrence and requires precisely the right conditions for it to occur. But how do fossils form and how much information can they reveal to us about organisms that lived in the past?

Aim

To investigate how fossils are formed and what they can reveal about organisms that lived in the past

You will need

- 4 fossil samples (e.g. fossilised coral, fossil footprint, trilobite, ammonite, shark's tooth, leaf fossil)
- Reference material with information on fossils
- A hand lens (one per group)

What to do

1. For each of the fossils that you have been given, complete as many observations as possible and record them. Your observations should include the following:
   - Sketch of fossil
   - Name of organism
   - Phylum or classification
   - Location or habitat where fossil was found
   - Type of rock in which fossil was found.
2. Examine the individual fossil specimens carefully and attempt to classify them into the phylum (or class or order if possible) to which they belong.
3. Using a hand lens, examine the rock surrounding the fossil specimen and try to identify the type of material it is. Check to see if the information provided with the fossils gives you any insight into what the material might be.
4. Sketch the fossil specimen and note which parts have been preserved and which have not.

What did you discover?

1. Deduce and explain how each of the fossils has been preserved. Compare the material the fossil is made of with the original living tissue. Explain how the two are different and how the composition of the fossil may have come about.
2. Compare the fossilised specimens with similar species that exist today, and identify which parts have been preserved and which parts have disappeared. Explain why this would be the case.
3. Explain how much information scientists can gain about an animal from a single fossilised tooth. Investigate this topic on the Internet, using the example of *Carcharodon megalodon* (an extinct shark) as the focus of your research. Find out why this particular example of animal reconstruction has a controversial history.

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8.1 INVESTIGATION

Homologous structures

Charles Darwin noted that many animals share similarities in body structure. He argued that this seemed to suggest that the structures had developed from a common ancestral form. Is this explanation of the similarities in structures as obvious as he suggested?

Aim

To investigate homologous structures in the pentadactyl limb of various vertebrates

You will need

Four examples of vertebrate pentadactyl limb. These could be actual skeletons, models, photographs or illustrations of the limbs (e.g. frog, bird, dolphin, dog, cat).

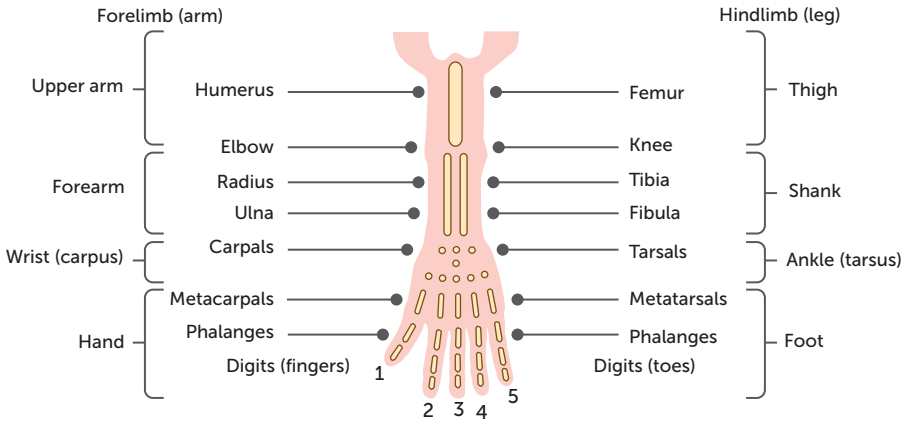

FIGURE 8.28 A generalised pentadactyl limb

What to do

For each of the samples that you have been given, complete as many observations as possible and note them in your results.

1. Carefully examine the forelimbs and hind limbs of the generalised pentadactyl limb (Figure 8.28) and of each specimen, and draw a quick sketch of each in your results section. Make a table and record the number of bones that make up each section of the forelimbs and another table for the hindlimbs. Include the hand/foot area, wrist/ankle area, forearm/shin area, and the upper arm/thigh area.
2. Describe any other differences that you may have observed for each specimen when it was compared to the generalised diagram of a pentadactyl limb.

Results

Your results should include:

- Name of organism
- Sketch of forelimb
- Sketch of hindlimb
- Summary table of counts
- Descriptions of differences

Discussion

1. Analyse how the numbers of bones in each area of your specimens compares with those of the generalised pentadactyl limb.
2. Other than bone numbers, explain what other differences you find in the limb structures compared with the generalised pentadactyl limb.
3. Suggest and explain reasons for the differences noted for each particular animal.
4. Suggest what advantages these differences might offer to the species concerned.
5. Identify the basic similarities in the different limbs and explain why these might be found in so many different species, even though they may occupy a variety of different habitats.

Discussion

Write a summary of your analysis. What is your conclusion?

Taking it further

Use the Internet to examine the limb structures of other animals to see how they compare with the ones you have examined in this activity. Are you able to see how closely animals are related to one another by their similarities?

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8.2 INVESTIGATION

Hominid skull analysis

Aim

In this investigation, you will analyse various hominid/primate skulls. This is an excellent opportunity to explore various anatomical adaptations that have diverged in hominids over the course of their evolution.

Time requirement

45 minutes

Materials

- *Pan troglodytes* (chimpanzee) (Modern)
- *Gorilla gorilla* (gorilla) (Modern)
- *Homo sapiens* (human) (Modern)
- *Homo neanderthalensis* (Neanderthal human) (120 000–30 000 years ago)
- *Homo erectus* (upright human) (2.0 mya)
- *Australopithecus boisei* (2.3–1.2 mya)
- *Australopithecus afarensis* ('Lucy') (4.0 mya)
- Tape measure (in millimetres)

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Skulls may have sharp edges.	Handle with care and do not run fingers over skull teeth.

Procedure – Examining the braincase

1. Examine the frontal bone (forehead) of each of the skulls and determine whether they appear more vertical or flat. Ensure the eye sockets are oriented forward while doing this.

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2 Examine above the orbital and determine whether a supraorbital (brow ridge) is present. If so, see whether the brow ridge is continuous or divided in the middle.
3 Measure the width of the braincase at the widest point. Make all measurements in millimetres.
4 Look for evidence of a sagittal crest running lengthwise along the midline of the top of the skull. Identify whether it is prominent, present or absent.
5 Measure the distance between the front teeth and the front ridge of the foramen magnum.
6 Examine behind the ear of the skull and determine whether the mastoid process is fairly flat or noticeably protruding.
7 Draw up a copy of Table 8.5 and record the results of your observations in it.

Procedure – Examining the facial structure

1 Position the skull so that it is facing you. Examine the nasal bones. Identify whether they are flat or protruding.
2 Measure the maximum breadth (width) of the nasal opening.
3 Measure the maximum height of the nasal opening.
4 Starting at the outside of the upper back molars, measure the width of the maxilla (the upper jaw).
5 The bizygomatic breadth is the width of the face from the widest part of one zygomatic arch (cheek bone) to the widest part of the other zygomatic arch. Measure this distance.
6 Draw up a copy of Table 8.6 and record the results of your observations in it.

Procedure – Examining the dentition (teeth)

1 Examine the dental arcade (the shape made by the rows of teeth in the upper jaw). Observe the teeth towards the back and identify whether the teeth on each side of the jaw are parallel or diverging.
2 Reposition the skull so that you are viewing it from the side. Examine the incisors and identify whether they are vertical or angled forward.
3 Measure the width of the incisors on the left side of the jaw and then measure that of the incisors on the right side of the jaw. Add the width of all incisors together to get the combined width.
4 Examine the maxilla (upper jaw) and mandible (lower jaw) together. Identify whether the canine teeth project above or below the chewing surfaces of the other teeth.
5 See if you can identify the canine diastema (gap on the medial side of the canine – i.e. on the side nearer the middle of the body).
6 Measure from the back of the last molar to the front of the first premolar on the left side of the jaw. This will give you a measurement for the chewing surface of the teeth.
7 Draw up a copy of Table 8.7 and record the results of your observations in it.

Results

TABLE 8.5 Examining the braincase

SPECIMEN	FOREHEAD	BROW RIDGE	BRAINCASE	SAGITTAL CREST	FORAMEN MAGNUM	MASTOID

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TABLE 8.6 Examining the facial structure

SPECIMEN	NASAL BONES	NASAL OPENING WIDTH	NASAL OPENING HEIGHT	MAXILLA WIDTH	BIZYGOMATIC BREADTH

TABLE 8.7 Examining the dentition (teeth)

SPECIMEN	DENTAL ARCADE	INCISORS ANGLE	INCISORS WIDTH	CANINE	DIASTEMA	CHEWING SURFACE

Based on the data you have collected, draw a sketch of one characteristic (e.g. presence of brow ridge) for each specimen, with the sketches arranged in order from great apes to modern humans, so that you can see any trends over evolutionary time.

Discussion

1. The canine teeth have drastically reduced in size from great apes to modern humans. Explain why this might be.
2. Explain why the face has become progressively flatter over the evolution of hominids.
3. Describe how the position of the foramen magnum relates to body posture and locomotion.
4. Certain areas of the braincase enlarged before others in our evolution. Describe how the various areas have enlarged over the period of our evolution.
5. What traits differentiate modern apes and modern humans?
6. Using your measurements and the facial features you observed as evidence, are modern humans or modern apes more closely related to extinct hominids?
7. Imagine you found the remains of a skull that only contained the mandible. Would that be enough evidence to determine whether it belonged to a modern human, an early hominid, or an ape? Explain your answer.

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CHAPTER 8 SUMMARY

Chapter 8 Activity sheet

- Life has existed on Earth for approximately 3.5 billion years and has changed over time. Some changes in species are rapid and occur after a period of stasis (punctuated equilibrium). Most changes take place over long periods of time (gradualism).
- Darwin's theory of evolution by natural selection replaced Lamarck's theory of transmutation of species.
- Evidence for evolution comes from five main areas of study: biogeography, comparative genomics, the fossil record, comparative embryology and comparative anatomy.
- The positions of landmasses are in constant change. Geologic and fossil evidence tell us that 200 mya a single supercontinent – Pangaea – existed, which later separated into smaller landmasses.
- Biogeography is the study of the distribution of organisms and ecosystems across the world and through geologic time.
- Different organisms share molecular and structural homologies. The DNA present in all organisms indicates that modern life descended from a single population of organisms.
- Comparative genomics provides evidence for the theory of evolution and helps us map the degree of species relatedness.
- Comparative genomics and comparative biochemistry are possible due to bioinformatics, which is the computer analysis of large volumes of biological data.
- Evolutionary relationships between groups can be represented using phylogenetic trees. Analysis of phylogenetic trees gives us insight into how closely related species are.
- The fossil record provides evidence of extinct organisms. That change has occurred in species and in groups of species over long periods of time is evidenced by fossils, as well as by the progression of simple to more complex organisms in the fossil record.
- Comparative dating is used to determine the relative age of a rock or fossil. Absolute (or chronometric) dating provides the actual (approximate) age of a fossil or rock.
- Comparative anatomy is used to establish evolutionary relationships on the basis of structural similarities and differences, including the comparative study of embryos.
- Evolution can be classified as convergent or divergent. Analogous structures provide some evidence for convergent evolution, and homologous structures provide some evidence for divergent evolution.

CHAPTER 8 GLOSSARY

Absolute dating The process of determining the age of rocks and the fossils they contain on the basis of the physical or chemical properties of materials in the rock

Adaptation An evolved structural, physiological or behavioural characteristic of an organism that increases its chances of survival and reproduction in a particular environment

Adaptive radiation The process by which a species rapidly diversifies into many taxa with differing adaptations; it can be triggered by many factors, such as the emergence of reproductive barriers within a population, changes in the availability of resources, new challenges or new opportunities; it is a type of divergent evolution

Analogous structure Features of organisms that have the same function but not the same structure

Ancestor A species from which other species have evolved

Biogeography The study of the distributions of living things over a geographical area and how those distributions have changed over geologic time

Bioinformatics The digital storage, retrieval, organisation and analysis of a large volume of

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biological data; bioinformatics has dramatically increased the size, accuracy and scope of data sets, such as those needed for comparative genomics

Clade A group of organisms that includes all the descendants of a common ancestor and the ancestor itself; for example, birds, dinosaurs, crocodiles and their common ancestor form a clade

Common ancestor An ancestor that is shared by different species

Comparative anatomy The study of the similarities and differences in structure between different organisms; a larger number of similar features indicates a more recent common ancestor

Comparative biochemistry Analysis of the similarities and differences in the cellular chemistry of different species; it particularly includes the study of proteins (especially enzymes) and the DNA that encodes them; the results enable evolutionary biologists to obtain a measure of the relatedness between species

Comparative dating The process of determining the age of rocks and their contained fossils relative to one another, allowing an estimation of 'oldest to youngest', without assigning an actual age in years

Comparative genomics A field of biological research in which scientists use a variety of tools to compare the genome sequences of different species; the more similar in sequence the genes and genomes of two species are, the more closely related those species are

Conserved Refers to DNA or protein sequences that have been preserved by natural selection and are still the same or very similar in different species

Continental drift The relative movement of Earth's continental landmasses, which appear to drift over Earth's mantle

Convergent evolution A process whereby unrelated organisms evolve similar adaptations in response to a similarity in their environments

Divergent evolution A process whereby related species evolve new traits over time spent living in different habitats, becoming increasingly different from the common ancestor and from one another, giving rise to new species

DNA–DNA hybridisation A method used to analyse relatedness; similarities in the base-pairing of DNA strands are analysed to show evolutionary links between organisms

Embryology The study of the anatomy of embryos and how they develop over time until the adult stage

Eon A major division of geologic time that is itself divided into eras

Epoch A division of geologic time (periods) that is marked by one or more significant events

Era A division of geologic time (a subdivision of eons) that is itself divided into periods

Evolution The process of cumulative, gradual, inheritable change in a population of organisms that occurs over many generations and a relatively long time

Fossil Preserved remains or traces of an organism

Genomics The study of the genome – how genes interact with one another and the environment, and the resultant proteins produced; knowledge of an organism's entire DNA sequence

Gradualism A theoretical model of evolution that proposes there has been a steady, slow divergence of lineages, irrespective of gaps in the fossil record

Homologous structure Feature that has the same general structure but different functions in different organisms

Homology The existence of shared ancestry between a pair of structures or between genes

Isotope Atoms of an element that have the same number of protons but different numbers of neutrons, and therefore different relative atomic masses

Molecular homology The identification of shared biomolecular elements – generally genes – used to test the closeness of relationships between organisms; it can demonstrate common ancestry

Molecular phylogeny The study of evolutionary relationships using comparative genomics

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Morphological Structural

Mutation rate The number of changes per gene copy in a population over a period of time

mya Millions of years ago

Niche An organism's habitat, way of life, or the way it functions in its environment

Palaeontology The study of life in the past, based on fossil remains

Period A division of an era of geological time that is itself divided into epochs

Phylogeny Evolutionary relationships that exist between species, often expressed in a tree-like diagram

Principle of superposition The principle that states that the oldest rock layer is found in the deepest position, and each consecutive layer above it is relatively younger; it indicates the *relative* ages of the rock layers and the fossils within them; this principle is fundamental to our interpretation of Earth's history

Punctuated equilibrium A theory of evolution that proposes new organisms evolve quickly after a long period of no change, rather than evolving by gradual change

Radiometric dating Uses the known rates of decay of naturally occurring radioactive isotopes present in a rock or fossil to obtain an absolute date for its age

Relatedness A measure of the evolutionary distance between two species; they are more related if they have a recent common ancestor and less related if they have a less recent common ancestor

Speciation The evolution of one or more new species from an ancestral species

Species A group of similar organisms capable of breeding and whose offspring are also fertile

Stratum (plural strata) The layers of rock in an area (profile); strata occur in order, with the oldest layers at the bottom

Taxon (plural taxa) A named group of organisms, such as beetles or reptiles

Theory A collection of models and concepts that explains specific systems or phenomena; scientific theories allow predictions to be made and hence are falsifiable

Vestigial structures Biological structures that have lost most, if not all, of their original function in the course of evolution; in ancestral organisms the structures served a purpose, but in their descendants the structures become atrophied or rudimentary

CHAPTER 8 REVIEW QUESTIONS

Remembering

1. List the categories of evidence for evolution.
2. Describe Lamarck's theory of transmutation of species.
3. Define:
   - a gradualism
   - b punctuated equilibrium
   - c biogeography
   - d vestigial structures
   - e homologous structures
   - f comparative genomics.
4. The fossil record is a vital source of evidence in the modern evolutionary synthesis, but it is patchy and incomplete. Outline the reasons for this patchy record.

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Understanding

5 The thylacine (marsupial) and the American gray wolf (placental) evolved independently of each other in widely separated biogeographic locations, but both animals had a similar appearance and occupied similar ecological niches. The similarities between the two organisms are most likely a result of which evolutionary pattern?

6 Birds and bats both have wings, whereas mice and crocodiles don't. Explain whether this means that birds and bats are more closely related to one another than they are to mice and crocodiles.

7 Forty per cent of the world's species of fruit flies are found on the islands of the Hawaiian archipelago.

- **a** Propose a reason why the Hawaiian archipelago might provide a suitable habitat for so many different species of fruit flies.
- **b** Explain how adaptive radiation may have been involved in the evolution of Hawaiian fruit flies.

8 The sugar glider and the flying squirrel have a similar appearance. Both have a flap of skin between the forelimbs and hind limbs that enables them to glide from branch to branch. The flying squirrel is a placental mammal found in the Northern Hemisphere, and the sugar glider is a marsupial found in Australia.

- **a** Name the process that has resulted in these species having similar features.
- **b** Name and describe the evolutionary pattern that accounts for the similarity of these two species.
- **c** Suggest how these two animals – one a placental and one a marsupial – would differ in other ways.

Applying

9 Embryological studies show bird embryos develop a fourth finger and a fifth toe that vanish as the foetus develops. This vestigial developmental structure is evidence for common descent.

- **a** Explain what this evidence explicitly says about the characteristics of the ancestors of birds.
- **b** Explain whether you would expect a complete fossil skeleton of a common ancestor showing this characteristic to have been found.

Analysing

10 New Zealand has no large native land mammals, but has been home to some highly specialised bird species. Many of these birds have lost the ability to fly: for example, the five species of Kiwi, which have developed some distinctive features. These features include mammal-like characteristics, such as a keen sense of smell, bone marrow (which makes bones heavy and unsuitable for flight) and a pair of functional ovaries in females (most birds have only one functional ovary), all of which are highly unusual for birds.

- **a** Using the information above, give examples of:
  - **i** divergent evolution
  - **ii** convergent evolution
  - **iii** analogous structures.
- **b** Would molecular homology studies show that the five species are more closely related to other birds or to mammals?

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Evaluating

11 The hoatzin (*Opisthocomus hoazin*) is a remarkable bird from South America. It has only one known fossil ancestor, a 10-million-year-old skull fragment found in Colombia. The age of the fossil demonstrates that hoatzins were endemic to South America, because the fossil pre-dates the land bridge between North and South America by 8 million years.

Genetic analysis of the living hoatzin shows it is unique, perhaps because of its extensive history of geographic isolation, and it has its own suborder. Chicks of the hoatzin show a characteristic shown in no other living bird: a pair of claws on their wings. A similar characteristic is seen on the bird-like dinosaur *Archaeopteryx*, which had three wing claws. From this data, give an example of each of the following types of evolutionary evidence.

- **a** palaeontology (the fossil record)
- **b** biogeography
- **c** morphology
- **d** genetics

12 The 1861 discovery of the Jurassic-age fossil skeleton of the feathered dinosaurian bird ancestor *Archaeopteryx* from Germany was a key moment in the development of Darwinian theory. The discovery of the pigeon-sized animal was brought to the attention of Charles Darwin, who commented that 'hardly any recent discovery shows more forcibly than this how little we as yet know of the former inhabitants of the world' (*The Origin of Species*).

The skeleton of *Archaeopteryx* clearly shows that it had claws on its forelimbs, well-developed feathers on its wings (allowing for weak, gliding flight), teeth and a long, bony tail.

- **a** Define transitional form.
- **b** Discuss the limitations of the evidence for the evolutionary relationships between dinosaurs, *Archaeopteryx* and birds. Which type of evidence can be used and which types cannot?

Creating

Use the following genetic and morphological data to answer questions 13–15.

Genetic characters

Sequence of portion of chloroplast DNA

Japanese black pine (*Pinus thunbergii*)	T A A T A A A G G A G G - - - - - - G A C T T A T G T C A C
Bhutan white pine (*Pinus bhutanica*)	T A A T A A A G G A G G G A - - - - - - C T T A T G T C G C
Chiapas pine (*Pinus chiapensis*)	T A A T A A A G G A G G G A C T T A G A C T T A T G T C A C
Eastern white pine (*Pinus strobus*)	T A A T A A A G G A G G G A C T T A G A C T T A T G T C A C
Lacebark pine (*Pinus bungeana*)	T A A T A A A G G A G G G A C - T G A - C T T A T G T C A C
Red pine (*Pinus resinosa*)	T A A T A A A G G A G G G A - - - - - - C T T A T G T C A C
Single-leaf pinyon (*Pinus monophylla*)	T A A T A A A G G A G G G A - - - - - - C T T A T G T C A C

Morphological characters

	Number of vascular bundles per needle	Sheath around needle bundle (1 = straight, 2 = curling back)	Number of needles per bundle	Seed wing (0 = absent, 1 = detachable, 2 =permanent)
Japanese black pine	2	1	2	2
Bhutan white pine	1	2	5	1
Chiapas pine	1	2	5	1
Eastern white pine	1	2	5	1
Lacebark pine	1	2	3	2
Red pine	2	1	2	2
Single-leaf pinyon	1	2	1	0

FIGURE 8.29 Genetic sequences

13 Construct a phylogenetic tree for the seven pine species, based solely on the morphological data.

14 Analyse the genetic sequences for the seven pine species and determine which two are the most closely related species.

15 Create an argument summarising whether you think comparative genomics or comparative anatomy (looking at morphological characters) provides more reliable evidence for relatedness, and why.

PRACTICE EXAM QUESTIONS

1 The early evolution and diversification of eukaryotes required increasing amounts of which of the following gases in the atmosphere?

A oxygen
B carbon dioxide
C hydrogen
D nitrogen

[Q17 2019 SCSA]

2 Which of the following is evidence for the process of evolution?

A The fossil record has many gaps.
B Earth is about 4.5 billion years old.
C All species share a genetic code.
D Interspecific hybrids are usually sterile.

[Q24 2019 SCSA]

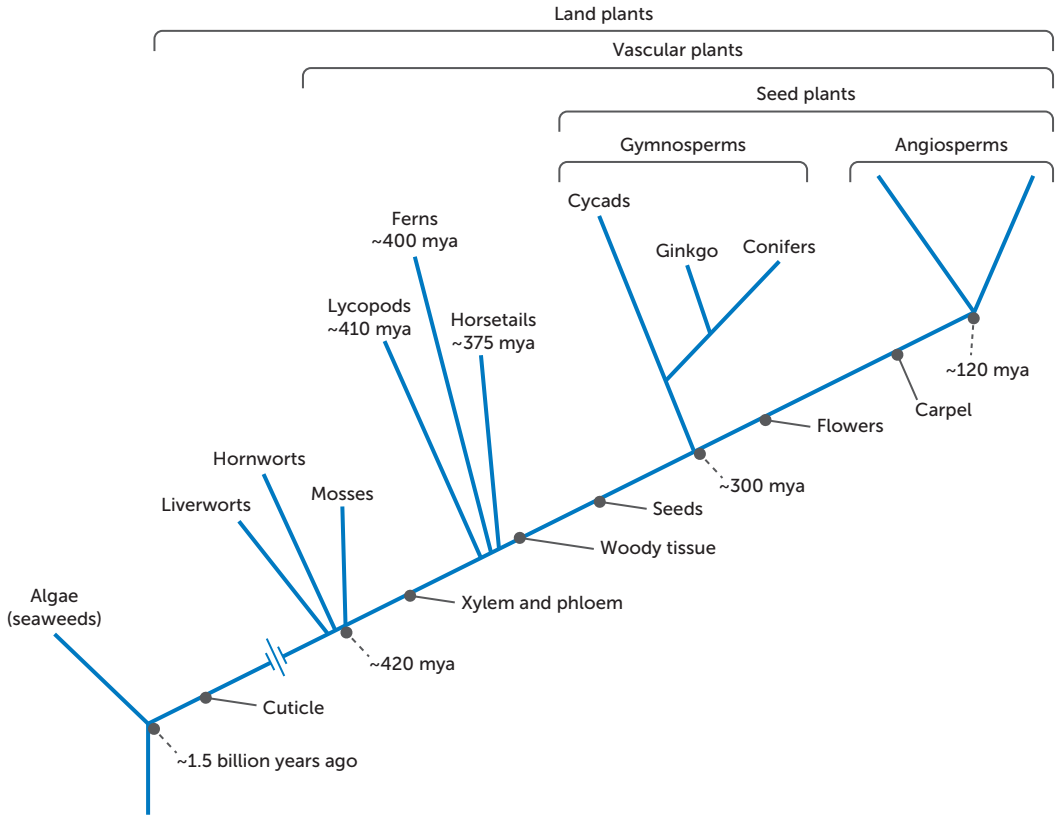
3 The first organisms on Earth were:

A eukaryotic and aerobic
B prokaryotic and aerobic
C eukaryotic and anaerobic
D prokaryotic and anaerobic.

[Q4 2018 SCSA]

Questions 4 and 5 relate to the information below.

The following phylogenetic tree shows the relationships among the major groups of plants and the points in their evolution at which particular characteristics arose. The time frame is in millions of years ago (mya).



4 The phylogenetic tree indicates that:

- **A** seeds evolved after flowers
- **B** woody tissue evolved after xylem and phloem
- **C** cycads have woody tissue and flowers
- **D** cuticle is present in ferns but not in mosses.

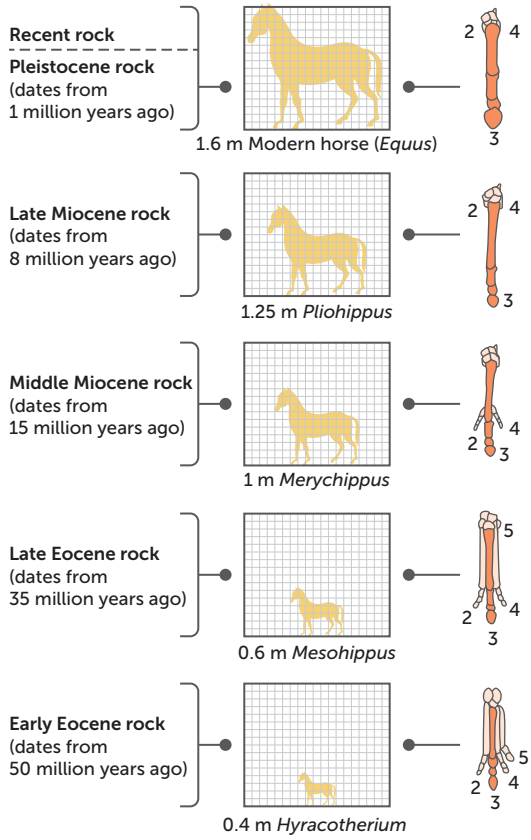
[Q14 2018 SCSA]

5 The phylogenetic tree also indicates that:

- **A** angiosperms evolved from mosses and ferns
- **B** gymnosperms evolved from angiosperms
- **C** liverworts, hornworts and mosses evolved from a single related group of plants
- **D** cycads, ginkgos and conifers evolved from a single related group of plants.

[Q15 2018 SCSA]

The following diagram shows the evolution of height and forefeet in modern horses and their extinct ancestors over the past 50 million years. The digits ('fingers') of the forefeet are labelled 2 to 5. Use the information to answer questions 6 and 7.



6 Describe the main features of the forefeet of the various types of horses that have appeared over the past 50 million years. (4 marks)

[Q32c 2018 SCSA]

7 Explain how biologists know about the evolution of the forefeet in horses over the past 50 million years. (4 marks)

[Q32d 2018 SCSA]

8 A biologist constructed a phylogenetic tree showing the evolutionary relationships among the Australian species of dung beetle. Explain how a phylogenetic tree can represent the evolutionary relationships between different species. (4 marks)

[Q31e 2019 SCSA]

9 Indicate the order in which the following life forms first evolved: eukaryotic cells, prokaryotic cells, land plants and marine animals. (4 marks)

First (oldest):
Second:
Third:
Fourth:

[Q34a 2017 SCSA]

10 Explain how fossils, comparative anatomy, comparative embryology and comparative genomics can each provide evidence for the theory of evolution. (10 marks)

[Q37b 2017 SCSA]

9 MECHANISMS OF EVOLUTION AND SPECIATION

CHAPTER 9 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 What do you think the term 'survival of the fittest' means?
2 Dogs and cats have a common ancestor, but have evolved very differently. Why have big cats evolved (such as the Siberian tiger) but not big dogs?
3 What are the mechanisms for evolution? If they are absent, can evolution occur?

SCIENCE UNDERSTANDING

- mutation is the ultimate source of genetic variation as it introduces new alleles into a population
- natural selection occurs when selection pressures in the environment confer a selective advantage on a specific phenotype to enhance its survival and reproduction; this results in changes in allele frequency in the gene pool of a population
- in addition to environmental selection pressures, sexual selection, mutation, gene flow and genetic drift can contribute to changes in allele frequency in a population gene pool
- speciation and macro-evolutionary changes result from an accumulation of micro-evolutionary changes over time
- selective breeding (artificial selection) through the intentional reproduction of individuals with desirable characteristics results in changes in allele frequencies in the gene pools over time
- differing selection pressures between geographically isolated populations may lead to allopatric speciation
- populations with reduced genetic diversity face increased risk of extinction

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

Getty Images/Max shen

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9.1 EVOLUTION AND ITS MECHANISMS

In the previous chapter, **evolution** was defined as a process that results in cumulative, **inheritable** changes in a **population**, spread over many generations. The **theory of evolution** states that all organisms have developed from previous organisms and that all living things have a common ancestor in some initial form of primitive life. It also states that all organisms are fundamentally similar because their basic chemistry was inherited from this very first organism. The evidence supporting the theory is significant, but unable to support all changes, including the initial change of the inanimate to the animate, and therefore evolution remains a theory. Since the 1930s and 1940s, the concept of evolution has 'evolved' to include genetics, and it is now understood to be due to change in the frequency of alleles within a **gene pool**. This understanding is the **modern synthesis** theory of evolution. If the basis of evolution is change, the next question is, what mechanisms bring about this change? The Hardy–Weinberg equilibrium principle says that allele frequencies in a population will remain constant in the absence of the four factors (mechanisms) that could change them. Those mechanisms are **mutation**, **natural selection**, **genetic drift**, and migration (enabling **gene flow**).

Mechanisms of evolution and natural selection
Watch this introductory video on the mechanisms of evolution. The mechanisms described explain how changes occur in a population.

9.2 A MECHANISM FOR EVOLUTION: MUTATION

Mutation is a source of new alleles in a population's gene pool. A mutation is a permanent change in the DNA sequence of a gene. A mutation can change one allele into another, and the net effect is a change in the frequency of an existing allele. The change in allele frequency resulting from mutations is small, so its effect on evolution is insignificant unless it provides a beneficial trait with respect to a particular **selection pressure** in the environment. A selection pressure is an abiotic or biotic environmental factor that enhances the survival and reproduction of those individuals in a population who possess a beneficial trait, and reduces the survival and reproduction of those individuals without that trait. It can contribute to changes in allele frequencies in a population gene pool and therefore also drive natural selection. When individuals in a population possess certain alleles or traits that are better suited to survive selective pressures, reproduce and pass on the advantageous alleles. This is known as 'survival of the fittest'.

A mutation may produce an allele that is selected against, selected for, or selectively neutral. Harmful mutations are removed from the population by selection and will generally only be found in very low frequencies equal to the mutation rate. Beneficial mutations will spread through the population over generations, through selection. That initial spread is slow, and is directly related to a population's reproductive rate. Whether or not a mutation is beneficial or harmful is determined by whether it helps an organism survive to sexual maturity and reproduce. It should be noted that if a mutation is beneficial, and selected for by the environment, it is the ultimate source of genetic **variation** in all populations. New alleles enter a gene pool, changing the frequency of alleles at the time of the mutation and after each new generation. Therefore, mutation is a mechanism for evolution.

The peppered moth, *Biston betularia,* is widespread in Britain (Figure 9.1, page 294). Historically, the standard moth form, *typica*, was white, liberally speckled with black. During the 1800s, British cities and the countryside were transformed by the Industrial Revolution. Hundreds of coal-powered factories produced large quantities of airborne soot and other pollutants. By 1895, 95% of moths in industrial regions of Britain, such as Manchester, were black (form *carbonaria*). A well-known lepidopterist (person who studies moths and butterflies), J.W. Tutt, proposed an evolutionary link between the Industrial Revolution and the moth population. Dark pigmentation was part of the natural, inheritable variation of the *B. betularia* population, but very rare. Blackening of tree trunks by

DNA mutation simulation
A reminder of what a mutation is and how different mutations can affect a protein. The protein product affects a phenotype ('trait'). Mutation is a method of introducing a new allele, which means a new protein, which means potentially a new trait.

Evolution of the peppered moth
Explore the evolutionary story of the peppered moth.

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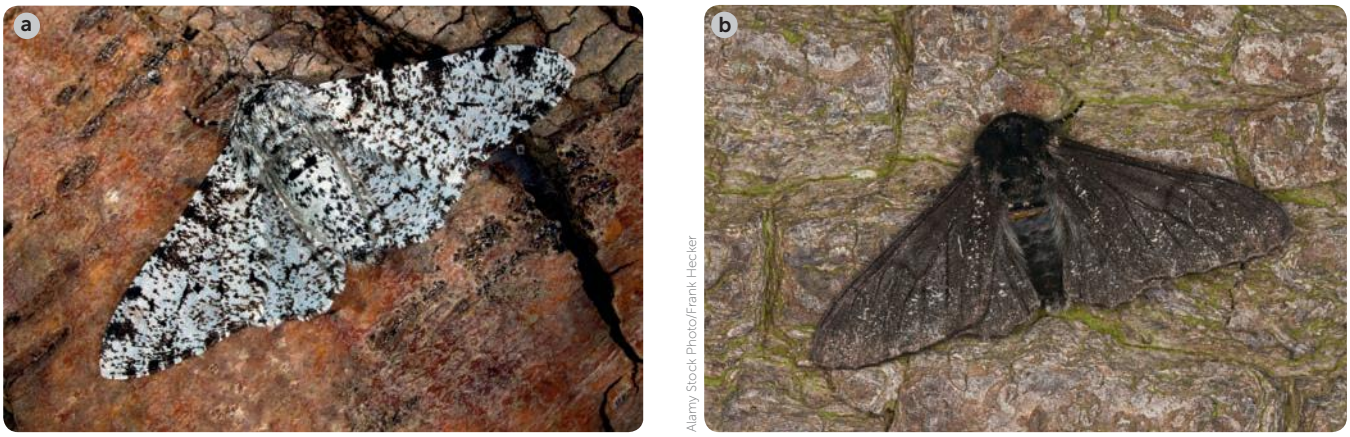
soot presented a new environmental pressure for the moth population. The dark-coloured moths were better able to evade bird predation than the common white speckled form. Over time, black moths came to dominate the population.

Since 1950, when clean air legislation was passed in Britain, the situation has been reversing; once again dark-coloured moths are suffering greater predation on the naturally white tree trunks, and their presence in the population is less common. Both dark and white forms continue to exist in the population.



Alamy Stock Photo/Nearby

Alamy Stock Photo/Frank Hecker

FIGURE 9.1 The peppered moth, *Biston betularia*, has **a** a white speckled *typica* form and **b** a dark *carbonaria* form.

Variation in populations

Variations in populations can be very small, but they are the basis of evolution. As in the peppered moth example, individuals in any population express a range of different phenotypes. A population is a group of individuals of the same **species** that live in the same geographic area and interbreed to produce fertile offspring. Members of a population have variation in their genotypes that causes variation in their phenotypes. Variation therefore is based on differences in DNA sequences, which give rise to different forms of genes (alleles), which in turn result in different phenotypes.

Evolution relies on genetic variation that is inheritable: it can be passed to the next generation and under certain circumstances may give an individual an advantage in survival and reproduction, compared with the rest of the population. In the case of the peppered moth, a mutation in the genotype produced a dark-coloured form in this population. This dark phenotype conferred a survival advantage in the changed environment. In a different environment, the same genotypic variation may give a disadvantage or have no effect at all. Either way, genetic mutation introduces new alleles and, therefore, additional variation into populations.

Gene pools

Genes are the means of transmitting phenotypes from one generation to another. Many genes can exist in different forms as alleles, and the characteristics of individuals are determined by the alleles they inherit. It is this variation in alleles carried by different individuals that leads to most of the variation in a population. The total collection of alleles within a population is referred to as the gene pool (see Figure 9.2).

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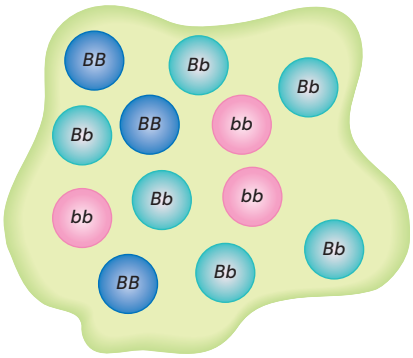

FIGURE 9.2 The sum of all the alleles found in a population is called the gene pool.

Key concept

Genetic mutations introduce new alleles into populations and are the ultimate source of variation. The sum total of all alleles present in a population is called the gene pool.

SCIENTIFIC LITERACY

Linking evolution, ecology and health

An understanding of evolution and ecology is essential when responding to threats to human health. For example, the Ebola virus, which has killed more than 11 000 people, has evolved quickly and is thought to have transferred from bat populations to human populations. Every transmission of the Ebola virus to a new host represents an opportunity for natural selection and therefore for evolution of the virus. Some strains have longer chains of transmission, with more mutations, enabling viruses to discover more fit phenotypes. Phylogenetic analyses revealed the great extent of evolutionary change that occurred early in the 2014 epidemic in West Africa, with 73 non-synonymous substitution mutations being found among 78 infected individuals just from Sierra Leone.

In 2018, an international body formed, known as the International Society for Evolution, Medicine, and Public Health (ISEMPH). The mission of ISEMPH is 'to foster communication among scientists, students, clinicians and public health professionals who want to use evolutionary insights to improve medical research and practice, and to use studies of human health and disease to advance evolutionary biology'.

The Tasmanian devil facial tumour disease is another example of ecology and evolution interacting to play a significant role in the health of a population. Tasmanian devils have been subject to this infectious and fatal cancer, and the species is under threat of extinction. However, it has been reported that some Tasmanian devils have developed resistance to the disease.

'These gene variants would have been around before, but there was no evolutionary advantage to them being at high frequency,' says Professor Katherine Belov of the University of Sydney. 'Since the arrival of this new disease, the animals without these variants would have been dying, leading to an increase in the frequency of these protective variants.'

Adapted from Charles L. Nunn, Susan C. Alberts, Craig R. McClain, Steven R. Meshnick, Todd J. Vision, Brian M. Wiegmann, Allen G. Rodrigo, 'Linking Evolution, Ecology, and Health', *TriCEM, BioScience*, Vol.65, Iss. 8. August 2015, pp 748–749.

Questions

1. Describe an ecological aspect of the Ebola disease.
2. Describe a health aspect of the Ebola disease.
3. Describe an evolutionary aspect of the Ebola disease.
4. Argue that an understanding of all three aspects is crucial in the management of the disease.
5. Explain how evolution is helping the Tasmanian devil.

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Question set 9.2

REMEMBERING

1 Define and give an example of each of the following:
   a mutation
   b variation
   c population.

UNDERSTANDING

2 Compare and contrast the types of variation in a population that results from mutation and crossing over.
3 'Mutations are the ultimate source of variation.' Explain the different ways in which sexual reproduction and mutation contribute to variation.
4 Explain why there are still examples of the white and dark form of *B. betularia* moths, even after clean air legislation has been passed.

Natural selection
Reinforce your understanding of natural selection by watching these videos.

9.3 A MECHANISM FOR EVOLUTION: NATURAL SELECTION

In 1868, two publications were released simultaneously through the Royal Society in London. These papers were by Charles Darwin and Alfred Russel Wallace. They outlined the authors' ideas on the evolution of life, what they referred to as **descent with modification**. This term highlights the important idea that all life that exists today has descended from shared ancestors. The shared characteristics observed in organisms led them to conclude that all organisms descended from an ancestor that lived in the past.

The mechanism proposed by Darwin and Wallace for how this happened is the process of natural selection, which can be used to explain many features observed in living things found in the world today. Through this mechanism, favourable traits are selected for and inherited, and become more common in subsequent generations. The process of certain traits gradually becoming more common over generations is known as **accumulation**.

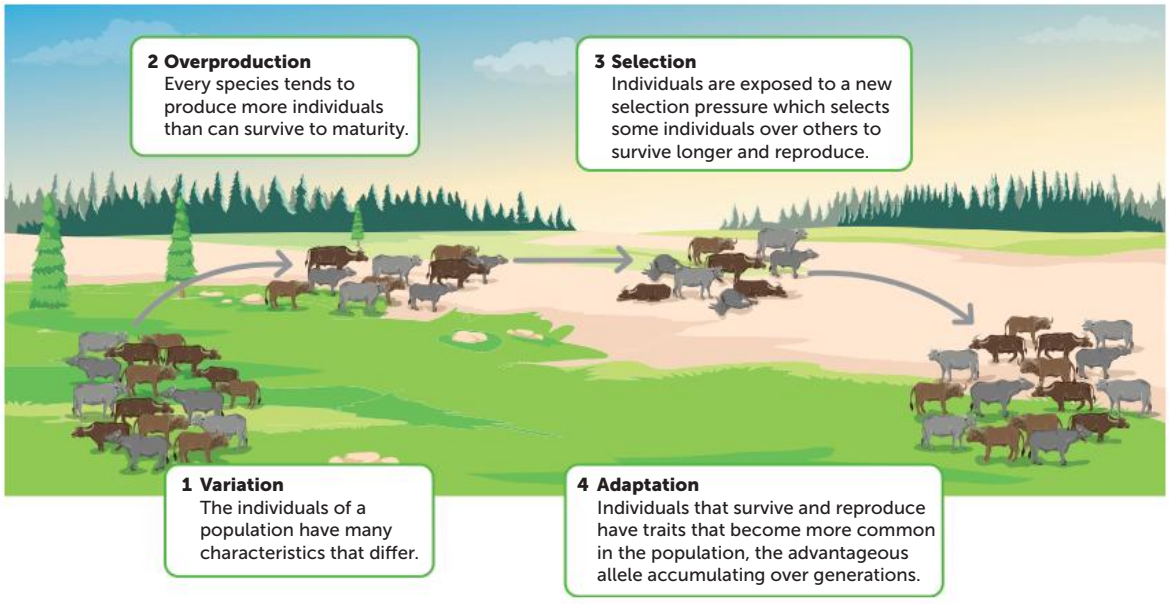

FIGURE 9.3 The theory of evolution by natural selection

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Natural selection occurs when selection pressures in the environment confer an advantage on a specific phenotype and enhance its survival and reproduction; this results in changes in allele frequencies in the gene pool of a population. Through this process, individuals that have certain inherited traits are more likely to survive and reproduce at higher rates than other individuals. This can cause changes in a population's allele frequencies and therefore is a mechanism for evolution.

Alleles are expressed in phenotypes, also known as traits. If a population possesses variation in a trait (different alleles for a gene), the population may experience natural selection. An inherited trait that allows an individual to survive and reproduce is called an adaptation. Depending on the environmental conditions, a phenotype may confer an advantage or a disadvantage to the individual, relative to individuals with other phenotypes in the population. If it is an advantage, then that individual will be more likely to survive and have offspring than individuals with the other phenotypes, and this will mean that the allele producing the phenotype will have greater representation in the next generation. If conditions remain the same, the offspring that are carrying the same allele will also benefit. Over time, the allele will increase in frequency in the population.

Natural selection only acts on the population's inheritable traits, selecting for beneficial alleles and thus increasing their frequency in the population, while selecting against deleterious alleles and thereby decreasing their frequency. Scientists call this process **adaptive evolution**.

TABLE 9.1 The principles of natural selection

PRINCIPLE	VISUAL AID
1 Variation Individuals in a population differ from one another; that is, individuals within populations show variation. Variation is due to mutation in alleles and meiosis/sexual reproduction processes. These processes include crossing over, independent assortment, random fertilisation, and random mating.	 Shutterstock.com/Dora Zett **FIGURE 9.4** Variation in the dog population
2 Overproduction There are more individuals produced in a population than the environment can support. Environmental resources are limited. Not all individuals can survive to reproduce. **Seahorse gives birth to 2000 babies** Watch this male seahorse give birth to thousands of baby seahorses. There are many, but only 5 in 1000 will survive.	 Alamy Stock Photo/Doug Perrine **FIGURE 9.5** Male seahorse 'giving birth' to many offspring. Not all will survive.

PRINCIPLE	VISUAL AID
3 Competition and survival of the fittest Environmental selection pressures such as food availability, predators and some diseases favour those with more advantageous traits or alleles. This may lead to competition between individuals in a population, and those with the advantageous trait may outcompete those without the advantageous trait. This is an example of 'survival of the fittest'. Those individuals who are more 'fit' are better suited to the environment in which the population lives. **Survival of the Tasmanian devil** Those who develop the trait of immunity against the transmissible devil facial tumour disease will be more likely to survive, reproduce and pass on their advantageous allele.	 Shutterstock.com/Chris Ison  Auscape. All rights reserved/ Dave Watts **FIGURE 9.6** Tasmanian devil: **a** with advantageous trait (fit); **b** without advantageous trait (not fit)
4 Higher reproductive rate Individuals with the inheritable advantageous trait are more likely to survive, reproduce and have a higher reproductive rate than those who do not possess the allele.	
5 Heritability Advantageous alleles are passed to offspring	One of the thorny devils' selection pressures is predation by wild birds and goannas. Advantageous traits they pass on to offspring are camouflaged colouration and large spines that serve as protection.  Shutterstock.com/Uwe Bergwitz **FIGURE 9.7** Thorny devil offspring showing advantageous traits of brown and gold colouration and large spines
6 Allele frequencies change over generations Over consecutive generations, the frequency of the advantageous alleles or traits increases. The frequency of the disadvantageous traits decreases. Over many generations (and usually a relatively long time), an advantageous allele can become fixed; that is, its frequency can become 100%. In contrast, the disadvantageous allele can become extinct (its frequency can become almost 0%).	

The driving force for adaptive evolutionary change is natural selection. Table 9.2 presents a case study for a hypothesis about long necks in giraffes being the result of natural selection using the principles of natural selection. How did giraffes develop such a long neck? If there was an inadequate supply of food, was this the selective force?

TABLE 9.2 The hypothesis that long necks in giraffes are the result of natural selection

PRINCIPLE	APPLICATION TO GIRAFFES
1 Variation	There was variation for short- and long-necked giraffes due to mutation and sexual reproduction processes.
2 Overproduction	More giraffes are produced than the environmental resources can sustain. There are not enough leaves to feed a whole population, and the last leaves to be eaten are up high.
3 Competition and survival of the fittest	The individuals in the giraffe population compete for the leaves high in the tree. Only those with the advantageous tall allele or trait can reach and consume enough food.
4 Higher reproductive rate	The individuals who possess the advantageous allele can survive and reproduce and have a higher reproductive rate than those who do not possess the advantageous allele.
5 Heritability	The giraffes with the higher reproductive rate are more likely to pass their inheritable, advantageous allele on to offspring.
6 Change in allele frequencies over generations	The next generation of giraffes have a higher frequency of advantageous (tall) alleles and a lower frequency of disadvantageous (short) alleles. Over many generations, the tall allele accumulates until the allele becomes fixed and the disadvantageous short allele becomes extinct.

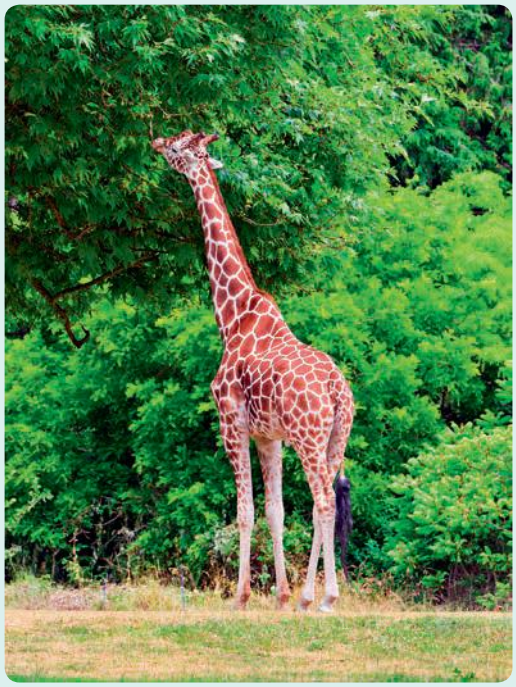


FIGURE 9.8 The allele to produce a long-necked phenotype suited the environmental pressures.

Key concept

Natural selection occurs when selection pressures in the environment confer an advantage on a specific phenotype to enhance its survival and reproduction; this results in changes in allele frequencies in the gene pool of a population. These advantageous traits are passed on to future generations and accumulate over time, resulting in a change in the gene pool of the population.

Artificial selection: animal and plant breeding

Darwin drew comparisons with breeding programs for domestic animals, including dogs and pigeons. The processes for breeding different strains of dogs and different varieties of pigeons were quite well understood (Darwin was an experienced pigeon breeder). Parental stock with certain desirable traits were selected and mated, and it was understood that these traits were often passed on to the offspring. Over time the new traits could be established in the populations. This process is called **artificial selection (selective breeding)**, and it relies upon human intervention to determine which traits are selected for.

Artificial selection is the intentional breeding of individuals with desirable traits, resulting in changes in allele frequencies in gene pools over time. The traits are beneficial to humans. The breeding for particular traits results in changes in allele frequencies over generations, and therefore this is a mechanism for evolution. Specific allele frequencies will decrease, and variation will also decrease, as humans breed for specific desirable traits. Artificial breeding has also been applied to sheep and cattle.

Sheep were very generic (had few breeds) and had random traits until about 300 years ago. Selective breeding has been practised since then to select for the best quality and quantity of meat, quality of wool, and size of the sheep. Sheep with the best characteristics were allowed to mate and produce offspring who would also have the favourable traits. Many different varieties of sheep have now been reared, all with traits that benefit humans.

Cattle, which currently have around 800 different breeds, have changed considerably from their wild ancestor, the auroch, which is now extinct. Cows have been bred for meat quality, or the quantity and quality of milk they produced. Jersey cows have been bred for both quantity and quality of milk. This type of selective breeding can pose problems for cows. Cows with large udders can be in discomfort due to the weight of the udder when it is full of milk.

The Belgian Blue is a breed of cattle that has been bred for the meat industry through artificial selection. The Belgian Blue's unusual physique comes from a naturally occurring 'double muscling' mutation. The mutation occurs in the myostatin gene (*M*), which codes for the protein myostatin ('myo' = muscle, 'statin' = stop). Due to the mutation, muscle development is not regulated, resulting in huge muscles.

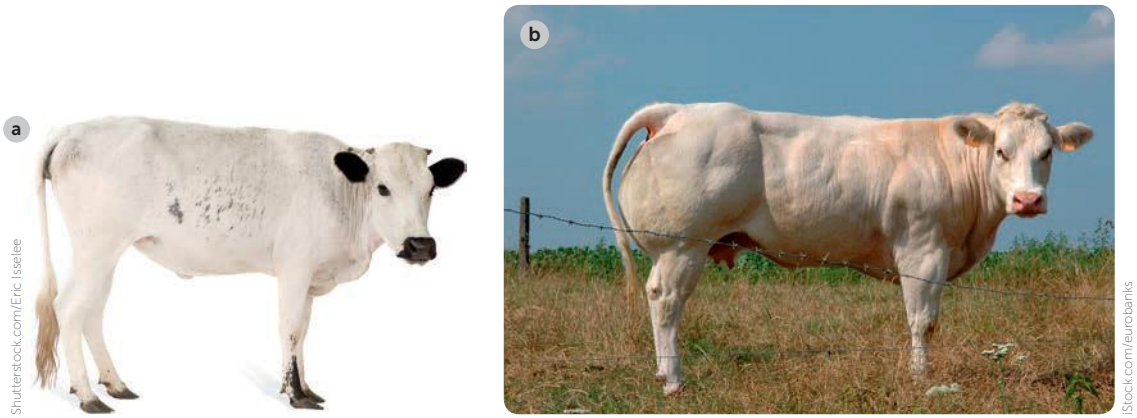


FIGURE 9.9 a A 'normal' cow; **b** a Belgian Blue cow

Artificial breeding has also been applied to many fruits and vegetables, such as corn, bananas and watermelons. Farmers and breeders use the practice of selection to cause major changes in the features of their crops and animals over the course of decades. The changes in allele frequencies cause evolution. The process is called artificial selection because people (instead of nature) select which organisms get to reproduce.

The teosinte plant, still found in South America, is the origin of today's corn plants. Native Americans practised selective breeding (artificially fertilising, inbreeding and crossbreeding teosinte) to create a higher yielding and tastier food source. The early corn plants had one cob per plant, on which there were at the most 4–5 kernels, and they were covered in an outer husk. Today's corn has exposed kernels and is significantly larger than the earlier varieties, with more cobs per plant.

Plant breeding has been practised for thousands of years. It is practised worldwide by individuals such as gardeners and farmers, and by professional plant breeders and researchers. It is conducted over many generations. International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought-resistant or regionally adapted to different environments and growing conditions.

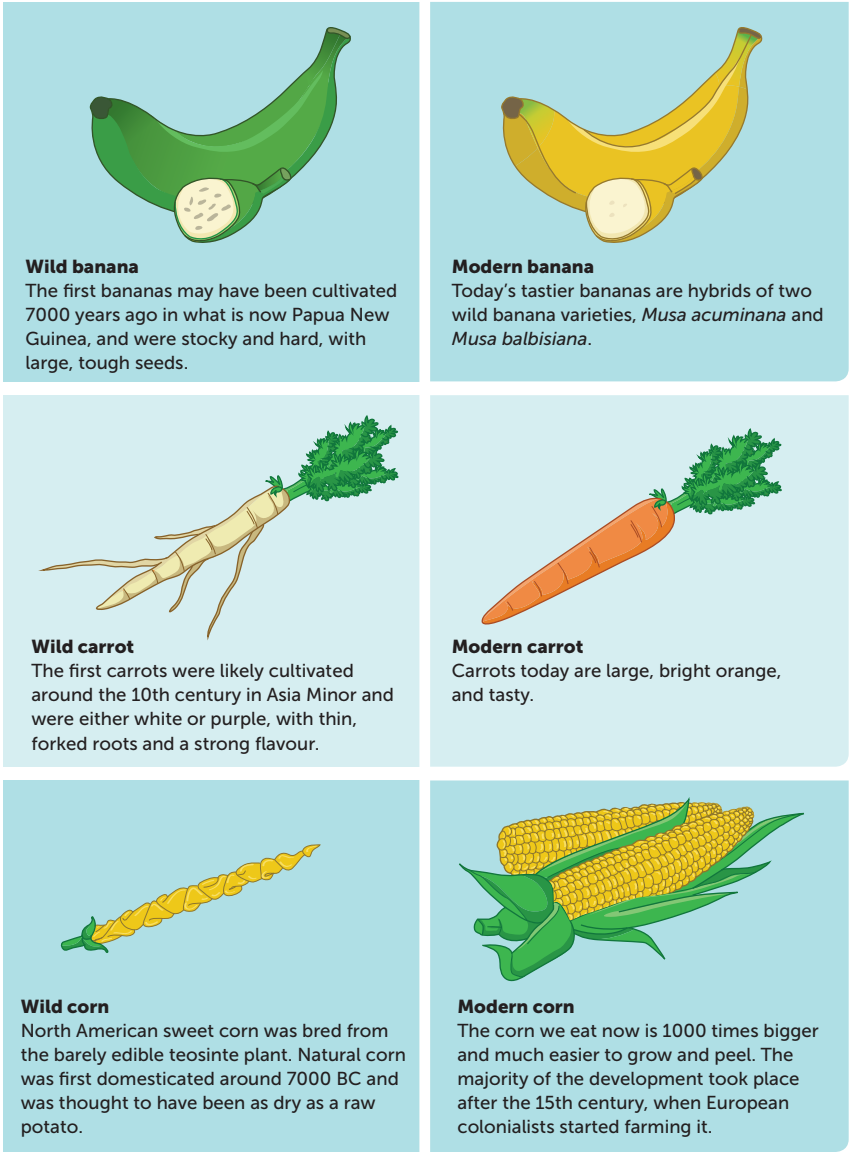
One major technique of plant breeding is selection, the process of selectively propagating plants with desirable characteristics and eliminating or 'culling' those with less desirable characteristics.

Another technique is the deliberate interbreeding (crossing) of closely or distantly related individuals to produce new crop varieties (hybrids) with desirable properties. Plants are crossbred to introduce traits or alleles from one variety or line into a new genetic background.

Traits that breeders have tried to incorporate into crop plants include:

- improved quality, such as increased nutrition, improved flavour, seedlessness or greater beauty
- increased yield
- increased tolerance of environmental pressures (salinity, extreme temperature, drought)
- resistance to viruses, fungi and bacteria
- increased tolerance to insect pests
- increased tolerance to herbicides
- longer storage period.

Artificial selection is similar to natural selection in that advantageous traits are passed on and allele frequencies change and may accumulate over generations. The major difference between artificial selection and natural selection is that humans choose traits that are advantageous to humans, whereas in natural selection, an environmental selection pressure selects a trait, and that trait is beneficial for the survival of the organism.



Wild banana
The first bananas may have been cultivated 7000 years ago in what is now Papua New Guinea, and were stocky and hard, with large, tough seeds.

Modern banana
Today's tastier bananas are hybrids of two wild banana varieties, *Musa acuminana* and *Musa balbisiana*.

Wild carrot
The first carrots were likely cultivated around the 10th century in Asia Minor and were either white or purple, with thin, forked roots and a strong flavour.

Modern carrot
Carrots today are large, bright orange, and tasty.

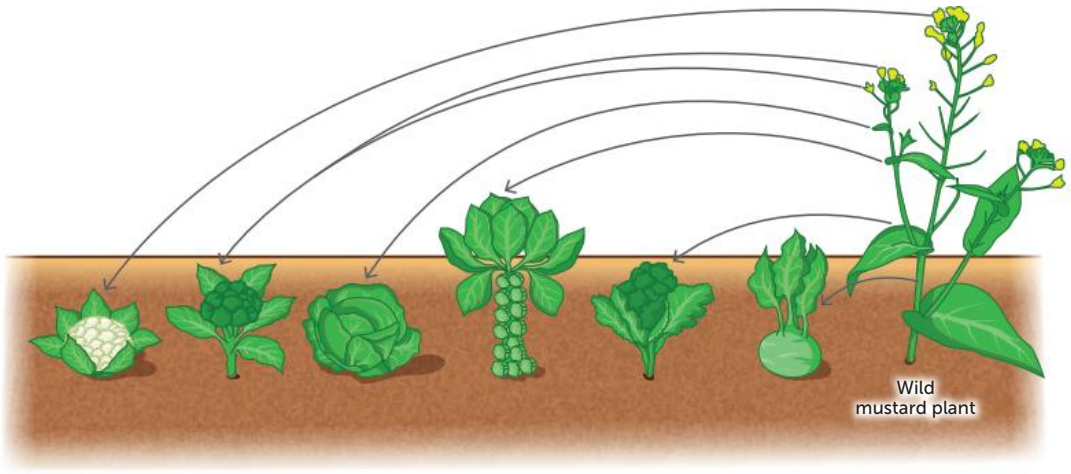
Wild corn
North American sweet corn was bred from the barely edible teosinte plant. Natural corn was first domesticated around 7000 BC and was thought to have been as dry as a raw potato.

Modern corn
The corn we eat now is 1000 times bigger and much easier to grow and peel. The majority of the development took place after the 15th century, when European colonialists started farming it.

FIGURE 9.10 Selective breeding of wild foods led to modern versions.

Artificial selection
Learn more about artificial selection here.

Selective breeding
Investigate selective breeding further and compare selective breeding with genetic engineering.



Cauliflower	Broccoli	Cabbage	Brussels sprouts	Kale	Kohlrabi	Strain
Flower buds	Flower buds and stem	Terminal leaf bud	Lateral leaf buds	Leaves	Stem	Modified trait

FIGURE 9.11 The wild mustard plant has evolved into different forms through selective breeding.

The similarities and differences of natural selection and selective breeding can be summarised in a Venn diagram.

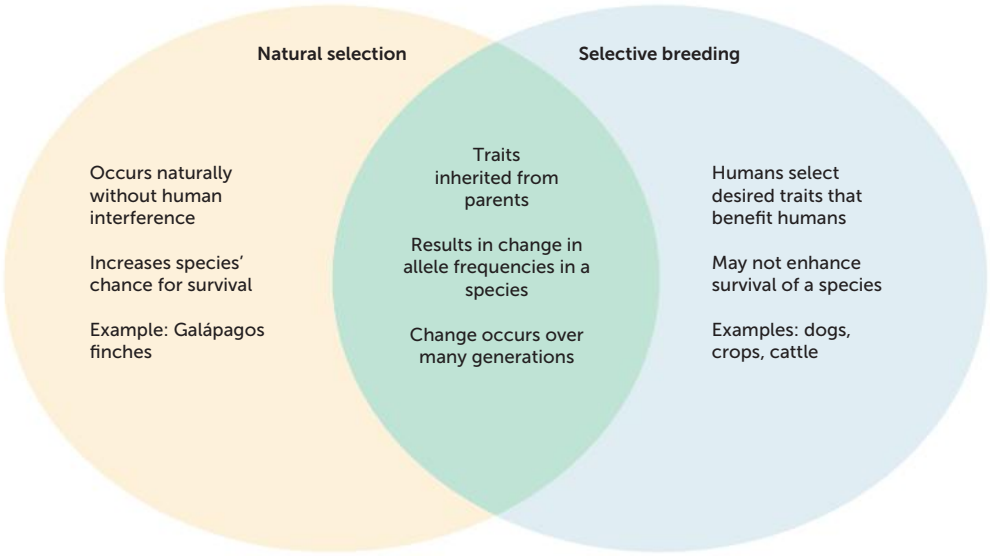


FIGURE 9.12 Venn diagram of the similarities and differences between natural selection and selective breeding

Table 9.3 compares the advantages of natural selection and artificial selection.

TABLE 9.3 Advantages of natural and artificial selection

NATURAL SELECTION	ARTIFICIAL SELECTION
Slower growth rate and therefore has time to adapt to changes in the environment, such as poor soil quality	Usually a faster growth rate
Higher genetic variation – less susceptible to changes in the environment. (Artificial selection usually breeds for one trait, which reduces variation, i.e. there is less chance of suitable alleles existing and more chance of extinction.)	Increased nutritional value, larger yield, pest resistance, drought resistance, disease resistance

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Sexual selection

Sexual selection is selection by male and female individuals of a population for an inherited trait that assists in copulation or in the winning of a mate. This type of natural selection is always linked to mating behaviour in animals. In this form of selection individuals with certain inherited characteristics or behaviours are more likely than others to obtain mates and pass on their genes.

Sexual selection can produce quite spectacular effects, such as the enormous antlers of a moose, or the long, showy tail of a male peacock. Over many generations, the frequency of the advantageous allele increases. Therefore, this process is a mechanism for evolution. It can lead to the fixation of advantageous alleles and the extinction of disadvantageous alleles. Although sexual selection is a type of natural selection, the advantageous trait does not necessarily assist the individual to survive its environmental selection pressure, it only helps it to win a mate.

Special characteristics such as the large tails of peacocks and lyrebirds, or the antlers of moose, are actually quite costly to the animal that is carrying them, and do not directly give them any extra survival advantage. In many cases, these attributes can be a threat to their survival. Loud and elaborate courtship displays attract predators as well as mates, and growing new antlers every year costs energy. So, what is the evolutionary advantage? One theory suggests that the females are selecting for a very obvious characteristic that correlates with other beneficial alleles. There have been some experiments carried out that suggest that this might be the case.

Sexual selection can also produce a phenomenon called **sexual dimorphism**. This term applies to species in which males and females have different appearances or size. Males and females of certain species are often quite different from one another in ways additional to their reproductive organs. Males are often larger, for example, and display many elaborate colours and adornments, like the peacock's tail, whereas females tend to be smaller and duller in decoration. Morphological difference such as this is termed dimorphism ('di' means 'two'): the two sexes of the same species exhibit two different forms.

Some examples of sexual selection can be surprising. For example, the Soay (*Ovis aries*) is a primitive breed of sheep that lives on the rocky islands off the coast of Scotland. It is well known for its agility on cliffs and for the large horns carried by many males. Large horn size appears to be a sexually selected characteristic and provides males with a significant advantage in securing mates. Variation in this trait appears to be controlled by a single gene. One allele (Ho^+) is linked to large horns and the other allele (Ho^p) to smaller horns.

Alamy Stock Photo/David McGill

FIGURE 9.13 Soay rams, showing their large horns

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Songs of the lyrebird
Explore with David Attenborough the repertoire of one bird.

ABC Catalyst
Watch the video on sexual selection (starts at the 12-minute mark and proceeds for 8 minutes).

Noisiest mating call
A male white bellbird screams a mating call. The birds further enhance their courtship by extending a black wattle, which grows from their jaws. Watch it here.

Mating dances
National Geographic peacock spider mating dances

Bird of paradise mating dance
National Geographic male bird of paradise mating dances

Sexual selection
Read more about sexual selection here.

Biologists have often hypothesised that sexual selection helps females somehow choose males who possess genes that confer a high level of **biological fitness**. But in the case of the Soay, they found that males with large horns actually have lower fitness overall. Rams with small horns have a better chance of surviving the harsh winters, and rams that are heterozygous, carrying one of each allele, are most successful overall in terms of survival and reproduction. This sexual selection ensures that the *Ho*^{p} allele survives in the population, even though it renders the rams less sexually fit.

The multimedia display of the lyrebird

The male lyrebird may not have the most spectacular tail in the world, so you might think it would not be attractive to females. But the bird's song display is a different story. The lyrebird is one of the most accomplished mimics in the animal kingdom. The males sing complex songs, mimicking animal and bird sounds, and even mechanical sounds, such as chainsaws. The males with a greater repertoire achieve better reproductive success.

iStock.com/CraigRJD

FIGURE 9.14 The male lyrebird displaying its tail

Key concept

Sexual selection is a form of selection in which individuals with certain inherited characteristics or behaviours are more likely than others to obtain mates and pass on their genes. The traits do not usually provide a benefit for survival.

Question set 9.3

REMEMBERING

1 Outline the meaning of the following and give an example of each.
   - **a** natural selection
   - **b** sexual selection
   - **c** artificial selection.

UNDERSTANDING

2 Describe how natural selection contributes to evolutionary change.
3 Identify the role of variation in evolutionary change.
4 All breeds of dogs originated from the wolf. Over time they have been bred for particular traits pleasing to humans, including friendliness. The cons of this breeding include back pain for dachshunds and breathing problems in pugs. Name the type of selection involved in dog breeding and discuss whether variation has increased or decreased in dogs.
5 In 2018, a man caught a sexually transmitted disease called gonorrhoea. He took antibiotics, but it was the first case of the infection that could not be cured with the first choice of antibiotics. The main antibiotic treatment – a combination of azithromycin and ceftriaxone – failed to treat the disease. The World Health Organization confirmed that this was a world first. The disease is caused by the bacterium called *Neisseria gonorrhoeae*, and the infection is spread by unprotected sex. Of those infected, about one in 10 heterosexual men and more than three-quarters of women and gay men have no easily recognisable symptoms. Apply the principles of natural selection to explain why the usual course of antibiotics did not work.

6 Male widowbirds have extremely long tails. Females prefer the longer tail when selecting a mate. Females have no outstandingly attractive features: they are inconspicuously mottled brown and have short tails. Suggest a reasonable explanation for the evolution of long tail feathers in male widowbirds.

9.4 A MECHANISM FOR EVOLUTION: GENETIC DRIFT

For variation to occur in phenotypes, more than one allele of a gene must exist. If there are multiple alleles, they can be affected by the random processes that occur in sexually reproducing organisms. Every reproductive event involves chance. This results in random changes in allele frequencies over generations. These random changes from generation to generation are known as genetic drift. Genetic drift is a mechanism of evolution in which allele frequencies of a population change over generations due to chance. Genetic drift occurs in all populations, but its effects are strongest in small populations.

There is usually a loss of genetic variation over generations. The smaller the population, the faster the fixation of alleles and the extinction of other alleles can occur. This means the process allows alleles in smaller populations to be eliminated faster than alleles in larger populations. Genetic drift is an important element of the evolutionary process. It occurs when a random, non-representative sample from a population produces the next generation. Thus, over time the proportion of an allele can 'drift' up or down. It is important to note that this is not due to any advantage or disadvantage associated with the allele (this is not a type of selection).

Each of us inherited half our alleles from our mother and half from our father. Which half of their alleles our respective parents passed on to us was a matter of chance. In addition to random fertilisation, each gamete has a randomly selected set of chromosomes due to random assortment of chromosomes during meiosis. In large populations, this randomness in inheritance of alleles is not noticeable overall. This is because the proportion of alleles that are affected is low. But if a population is small, there is a chance that some alleles present in a parental group will not be passed on at all. The same small number of alleles affected in a small population will be representative of a much higher proportion. These alleles may quickly be permanently lost from the gene pool. Alleles may be easy to lose, and they are virtually impossible to replace.

Genetic drift can be defined as the process of random changes in allele proportions within a population from one generation to the next.

Two extreme cases of genetic drift are:

- the **bottleneck effect**
- the **founder effect**.

Genetic drift can occur in a small population or when a large population is suddenly reduced due to a catastrophic event. This can give rise to a bottleneck effect. When a small group of individuals migrates and establishes a population in a new location, the founder effect may occur.

These effects are discussed in more detail below.

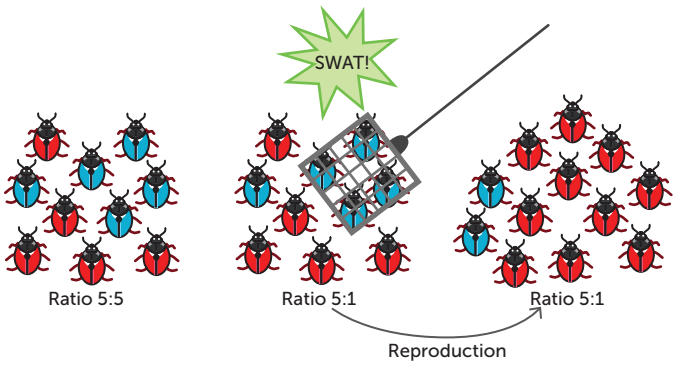

FIGURE 9.15 Genetic drift describes changes in allele frequency and proportions due to a chance event.

Bottleneck effect

Sometimes a catastrophic event or a period of adverse conditions drastically reduces the size of a population. In this scenario, certain alleles may be lost through chance. If some portion of the population survives the catastrophe, the original population gene pool cannot be recovered. The expanded population can only carry the alleles that existed in the population that survived the event. Therefore, the gene pool will now carry an indication of the bottleneck that occurred long after the population has recovered. The bottleneck effect occurs when there is a disaster of some sort that reduces a population to a small handful, which rarely represents the full genetic makeup of the initial population. This leaves smaller variation among the surviving individuals.

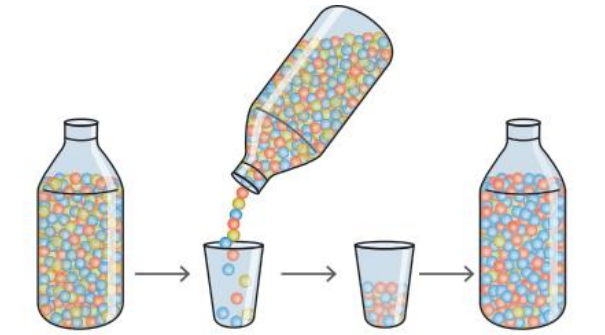

FIGURE 9.16 The bottleneck effect reduces genetic diversity in the population due to changes in the environment.

The bottleneck effect is an extreme case of genetic drift as a result of natural events or catastrophes.

Cheetahs are an endangered species that have survived a drastic genetic bottleneck. Facing a declining population, the surviving parents mated with their own offspring, and the resulting generations were left with strikingly low variation in alleles. One common allele is a mutated allele with negative effects on fertility. Typically, a male cheetah's sperm count is low, and 70% of the sperm are abnormal. Other common alleles result in lowered resistance to disease. Infections that are seldom life-threatening to other cat species can be lethal in cheetahs. There are only around 10 000 cheetahs left in the world today.

iStockphoto/DaleBHalbur

FIGURE 9.17 Cheetahs survived a severe bottleneck that increased the frequency of some unfavourable alleles.

A catastrophic decrease in population size can result in a loss of some alleles from the gene pool. This is the bottleneck effect. Deleterious genes can be preserved by chance.

Northern elephant seals have reduced genetic variation, most likely due to being hunted. Hunting reduced their population size to as few as 20 individuals at the end of the 19th century. Since then, their population has rebounded to over 30 000, but their genes still carry the evidence of their bottleneck. They have much less variation than a population of southern elephant seals that were not hunted.

The founder effect

The founder effect is a particular example of genetic drift. A few individuals who move to a new area and become isolated from a larger population might not carry all the alleles that were present in the original population. This means that the isolated population has less genetic

diversity than the original population, and deleterious recessive alleles may have a higher chance of coming together than they did in the original population. The founder effect happens when there is a dramatic decrease in genetic diversity caused by the development of small colonies of individuals, sourced from the original population, that remain isolated from other colonies. Only a small subset of the genetic diversity of the source population is likely to be included in the new population, and the relative frequencies of these alleles may be very different from what they were before.

The founder effect is an extreme case of genetic drift in a small population that migrates away from a large parent population, carrying with it an unrepresentative set of alleles.

This effect has been observed in human populations when small groups of particular religious or ethnic backgrounds have settled somewhere new and mixed very little with other populations. Around 200 people originally formed the Amish community of North America, and at least one of them harboured a recessive allele for Ellis–van Creveld syndrome. This syndrome, symptoms of which include dwarfism, polydactyly (extra toes or fingers) and sometimes a hole in the heart, has been common among Amish people of this region ever since.

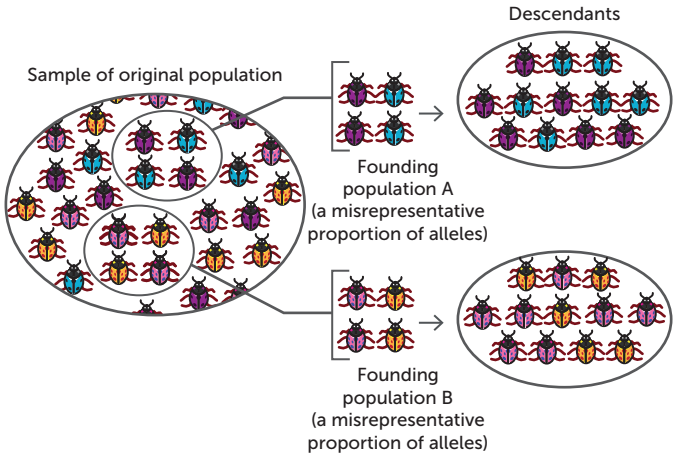

FIGURE 9.18 The founder effect reduces genetic diversity in a new population due to the reduced number and diversity of the founding individuals.

Key concept

Genetic drift is a mechanism of evolution in which allele frequencies of a population change over generations due to chance. Genetic drift occurs in all populations, but its effects are strongest in small populations. There is usually a loss of genetic variation over generations. The founder effect and bottlenecks are extreme examples of genetic drift.

Question set 9.4

REMEMBERING

1 Define:
   a founder effect
   b genetic drift.
2 Recall an example of a bottleneck effect.

UNDERSTANDING

3 Distinguish between a gene and an allele.
4 Outline why variations have to be inheritable for them to be relevant to evolutionary change.

9.5 A MECHANISM FOR EVOLUTION: GENE FLOW

Gene flow is the transfer of alleles, and it results from the migration of individuals from one population to another. This can be due to immigration (individuals joining a population) or emigration (individuals leaving a population). Populations, in a biological sense, are defined by their reproductive and genetic isolation. Few populations are completely isolated from others, and generally some migration takes place both into and out of a population. Gene flow may occur if the migrants breed. For example, immigrants may add new alleles to the gene pool and emigrants may completely remove some alleles or significantly change

their frequency. Many plants, for example, send their seeds far and wide, by wind or in the guts of animals; these seeds may introduce alleles common in the source population to another population in which they are rare. Gene flow is another mechanism for evolution, because the migration of individuals from population to population results in changes in the allele frequencies in populations.

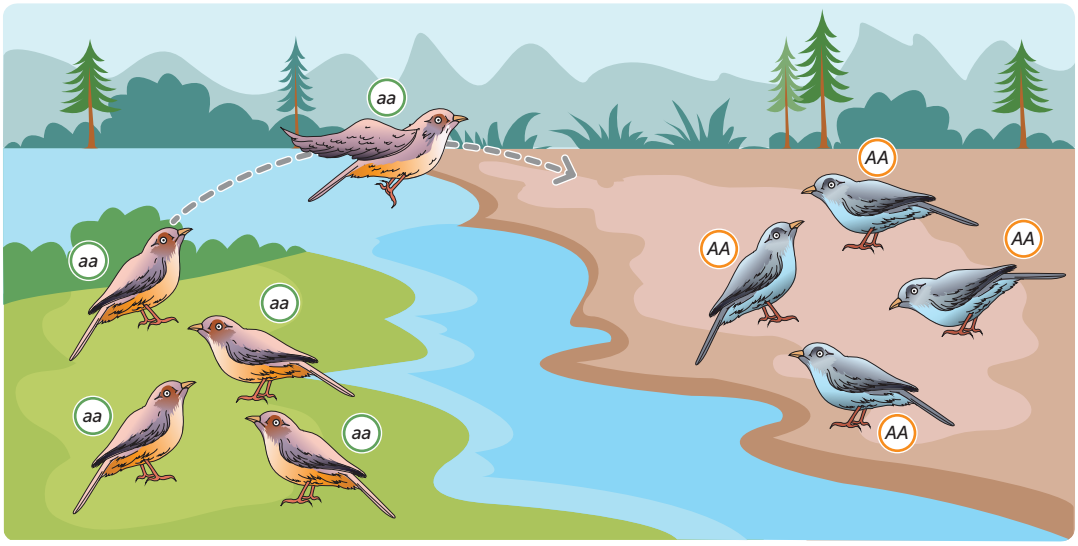

FIGURE 9.19 Gene flow is the transfer of alleles that results from the migration of individuals between populations.

Humans are polymorphic for a range of blood groups, including the ABO blood group. Indigenous Australians have some alleles that are present at frequencies different from those of other populations in the world. They have largely been isolated for the last 50 000 years, except for some gene flow from Asia and New Guinea in the northern regions of Australia. Most Indigenous Australians do not possess the I^B allele of the ABO blood group that results in either B or AB type blood. The I^B allele occurs at a frequency of up to 10% in European populations and up to 20% in Asian populations. The overall frequency of the I^B allele is increasing within the Indigenous Australian population due to the migration of people from Asia and Europe into Australia and the genetic flow between these populations.

Key concept

Gene flow is a mechanism for evolution due to migration. When an individual leaves one population and joins another population, it brings along its genetic information. When that individual breeds, its alleles are added to the new population.

Question set 9.5

REMEMBERING

1 Outline the meaning of 'gene flow'.
2 Differentiate between immigration and emigration.

UNDERSTANDING

3 Describe the mechanisms that can lead to changes in the gene pool of a population.
4 Outline how gene flow can affect allele frequency.

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9.6 THE BIGGER PICTURE OF EVOLUTION

The idea of adaptive evolution through natural selection is one of the most important biological concepts. Although Darwin and Wallace did not have a good understanding of the underlying causes of inheritance, they did realise that **variable traits** must be inheritable. Subsequent understanding of the inheritance of traits, initially through the work of Mendel, fitted perfectly with their theories to produce a combined theory referred to as the modern synthesis. The modern synthesis of evolutionary theory, sometimes referred to as neo-Darwinism, is one of the greatest refinements of a major theory to occur in biology.

Tree of Life

The Tree of Life project is a collaborative effort of biologists from around the world. It provides up-to-date information about biodiversity, the characteristics of different organisms and their evolutionary history (phylogeny).

The Tree of Life
Investigate the characteristics and evolution of various organisms.

Micro-evolution

The significant outcome of natural selection pressure is a change in the frequencies of various alleles within a population, a process called **micro-evolution**, which is change within a species. Micro-evolution refers to any small-scale change in the gene pool of a population. Changes in allele frequencies occur in a population over generations due to the mechanisms of mutation, natural selection, selective breeding, genetic drift and gene flow. The idea of micro-evolution puts the spotlight of evolutionary theory firmly on the genetic makeup of populations. We now see a population as a large pool of alleles that can change over time for a variety of reasons. Regardless of how this change is occurring, if the gene pool is changing over time, then evolution is occurring. This means genotype and phenotype frequencies are changing slightly over generations.

Macro-evolution

Major evolutionary changes above the species level are sometimes referred to as **macro-evolution**. **Speciation** and macro-evolutionary changes result from an accumulation of micro-evolutionary changes over many generations and over a very long time. Small-scale changes occur over one generation, but when there is a very long time scale (3.5 billion years of life on Earth), the micro-evolutionary changes accumulate into large changes. Large changes in a gene pool can be significant enough to lead to the production of a new species. This is known as speciation, as occurred in the Galápagos' finches over many generations and considerable time (see next section).

Macro-evolution includes the largest transformations in evolution. Examples are the changes that led to the evolution of mammals and of angiosperms (flowering plants). Macro-evolutionary patterns are generally what we see when we look at the large-scale history of life. Speciation is the link between micro-evolution and macro-evolution. Macro-evolution is the result of a series of speciation events.

Key concept

Micro-evolution is any change in the allele frequencies in a gene pool of a population over time. It occurs within species. Speciation is the production of a new species. Macro-evolution is the result of a series of speciation events.

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Question set 9.6

REMEMBERING

1 Define:
   a micro-evolution
   b macro-evolution.
2 Describe the link between micro-evolution and macro-evolution.

APPLYING

3 Construct a table summarising the different processes that can contribute to micro-evolution.

Speciation: an illustrated introduction
Watch the video on speciation as an introduction

9.7 SPECIATION

A species is a group of organisms that can interbreed to produce viable, fertile offspring and cannot breed with individuals of another species to produce fertile offspring. This is the **biological species concept** – a genetically isolated group with its own gene pool. The **morphological species concept** defines a species by its structural features. Individuals of the same species are morphologically similar.

Speciation is the formation of a new species. It is the process of one species splitting into two or more species.

The Galápagos Islands lie 1000 km west of Ecuador (South America) in the Pacific Ocean. When Darwin visited them in 1835, during his famous voyage on HMS *Beagle*, he realised that these islands were geologically quite young. They were teeming with life, but the animals and plants on the islands were of recent origin. Many of these appeared to be related to similar species on the South American mainland, but were also clearly different from them. One of the most famous groups of animals on the Galápagos Islands are the 15 or so species of giant tortoise, whose closest living relative, the Chaco tortoise, is found in mainland Argentina. Darwin wondered how they had got to the islands, and how they had changed into new species. He hypothesised that the tortoises on the islands originally came from the mainland population, but had changed over time to become better suited to the environment of the Galápagos.

Dreamstime/Rico Leffanta

Nature Picture Library/Gabriel Rojo

FIGURE 9.20 a The famous Galápagos tortoises are similar to **b** the much smaller Chaco tortoise (*Geochelone chilensis*), found in South America.

Scientists have hypothesised that there may be more than 8 million different species on Earth, but this is difficult to estimate accurately, because only around 1.2 million species have been identified and classified so far. Macro-evolution is a set of processes that attempts to explain how new species have evolved in such large numbers.

There are three broad processes that work together towards macro-evolution.

1 Natural selection favours phenotypes that make the population better adapted to its environment. Populations change over time as their gene pools accumulate small changes in response to natural selection. This is micro-evolution.

2 Eventually a population accumulates so many changes that a new species can be identified. This process can lead to speciation, the multiplication of species.
3 Sometimes a rapid series of speciation events leads to the development of a whole collection of new species, or even genera, families, or higher classification groups. This is referred to as macro-evolution.

Sometimes, the only evidence that a species existed is in the fossil record. When dealing with fossils only, the morphological species concept can be applied. This concept identifies different species based on their physical and physiological characteristics, but when used on the fossil record is limited to what can be observed. For example, red and grey kangaroos are two of Australia's most recognised marsupials. Kangaroos are quite well represented in the fossil record. Twenty-five mya, the ancestors of modern kangaroos lived in rainforests and fed on fruit. Kangaroos of today are connected to these distant ancestors through an unbroken line of descent.

Key concept

The biological species model defines a species as a reproductively isolated group of organisms. Species can also be identified through consistent differences in morphological and physiological traits, as well as genetic differences.

Kangaroo fossil?

9.1 APPLICATION

A fossil recently discovered in north-western Queensland has shed light on the evolution of the kangaroo. Read the weblink to find out more about this fossil and the information it provides for our understanding of kangaroo evolution.

New fossil discovery confirms evolution of kangaroo
Study more about the evolution of the kangaroo by reading this resource.

Mechanisms of speciation

Speciation occurs when a single population becomes two separate populations that are unable to interbreed due to changes that produce physical, biological or behavioural barriers. This separation, termed **reproductive isolation**, results in the gene pool of the original species being divided. Selection pressures act on the separated populations to cause micro-evolution, which can begin to change them in different ways. The accumulation of different phenotypes that match the different environments following reproductive isolation is known as **adaptive radiation**. Over time the allele frequencies of the separated populations may become so different that the individuals are no longer able to interbreed even if they are reunited, and we come to regard them as two distinct species. This is **divergent evolution**: one species has separated (diverged) into two separate species.

For example, small species such as frogs can cover long distances if enough time is available. Thus, during a period of hundreds of thousands of years, frogs can 'pond hop' hundreds of kilometres, which means that they can colonise new habitats and exploit new breeding sites. It seems that Victorian frogs colonised Tasmania in this way during the succession of recent ice ages. They did not evolve into new species until the subpopulations became isolated, in this case by the rising sea waters of an interglacial period.

Reproductive isolating mechanisms

Isolating mechanisms separate two groups and prevent them from producing fertile, viable offspring – that is, offspring that survive and can themselves reproduce. These mechanisms can operate before reproduction has occurred or after reproduction. Genetic isolation (in which populations become so genetically different that they can no longer interbreed) can occur before or after physical isolation. In either case, once isolation has occurred, the two groups can acquire different phenotypes, as natural selection works on the members of the two groups so they become adapted to their new, different environments (Figure 9.22, page 312).

FIGURE 9.21 Two Victorian frogs, **a** *Geocrinia victoriana* and **b** *Pseudophryne semimarmorata*, breed in the same habitat at the same time, but are prevented from interbreeding by alternating their calls so it is easier for females to distinguish them (a pre-reproductive isolating mechanism). If they do mate, their tadpoles do not develop (a post-reproductive isolating mechanism).

Pre-reproductive isolating mechanisms (pre-zygotic)

Some isolating biological or ecological mechanisms prevent organisms from being able to interact to reproduce. **Pre-reproductive isolating mechanisms** include the following:

- temporal (time) mechanisms: individuals breed during different seasons of the year or times of the day
- behavioural mechanisms: individuals have different courtship patterns
- morphological mechanisms: individuals have different reproductive structures, i.e. genitalia of different size, shape or location, so that mating is physically impossible.

The effectiveness of a geographic barrier as an isolating mechanism depends on the size and mobility of the individuals concerned. For example, small organisms may be easily transported across ocean barriers by being carried by other animals; parts of plants, such as seeds and stems, can float; small rodents can cling to floating vegetation carried by tides; and winds may carry insects over bodies of water.

Insects, in particular, can have very precise timing systems that determine when mating occurs. Periodical cicadas have one of the longest insect life cycles known.

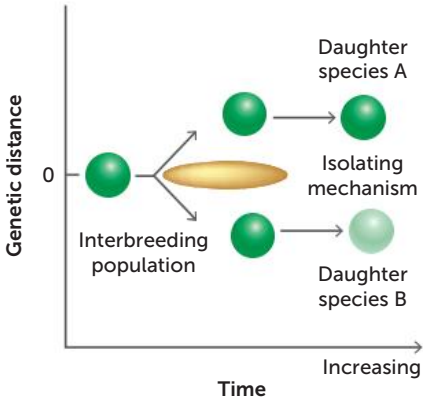


FIGURE 9.22 An isolating mechanism can prevent two subgroups of a species from breeding, until they are so genetically diverse that they form two new species.

FIGURE 9.23 *Magicicada,* a periodical cicada endemic to the northern United States

In North America there are several species of periodical cicadas (genus *Magicicada*), some that hatch out every 17 years and others that hatch every 13 years. Research has suggested the unusually lengthy life cycles may act to prevent different populations interbreeding and producing **hybrid offspring**.

Another example of a pre-reproductive isolating mechanism can be seen in frogs. The mating calls of frogs may sound very similar to us, but to other frogs they sound vastly different. Frogs usually reproduce only with members of their own species, so their call acts as a pre-reproductive isolating mechanism. In many cases, frogs have undergone speciation because their mating calls ensure that they mate only with frogs with the same call. If populations become isolated, and the call changes slightly, that can be enough to reproductively isolate the populations (even if they are reconnected) and prevent gene flow between them till they are genetically different enough not to be able to interbreed successfully.

Post-reproductive isolating mechanisms (post-zygotic)

If a frog does accidentally mate with a frog from another species, they will not produce fertile, viable offspring because the parents' chromosomes cannot line up successfully during meiosis, and no zygotes are formed.

Methods such as this are called **post-reproductive isolating mechanisms**. They do not prevent mating from occurring but they do prevent young from being produced. These genetic post-reproductive isolating mechanisms include the following:

- gamete mortality: the gametes do not survive
- zygote mortality: the zygote forms but does not survive
- hybrid sterility: adult offspring are formed but are infertile because they are unable to produce viable gametes, usually because of having received a different number or type of chromosomes from each species.

In general, hybrid sterility acts as a post-reproductive isolating mechanism in animals but not in plants. Many plants can interbreed; for example, polyploidy (multiple sets of chromosomes) is common in eucalypts. Species of coffee plants with 22, 44, 66 and 88 chromosomes are known; this suggests there was an ancestral plant with a haploid number of 11 and a diploid number of 22.

The key to the formation of new species involves reproductive isolation, leading to a disruption of the flow of genes, combined with selection pressures.

Allopatric speciation

In **allopatric speciation** (from the ancient Greek 'allos' = other and 'patra' = homeland), gene flow is disrupted when populations become physically separated through geographical isolation. The populations diverge. This may be because of different selection pressures acting on the two populations, or it may be due to other random processes such as genetic drift (see page 305). The isolation may happen on a very small scale, such as when a river or stream changes course and subdivides a population of small animals that can't cross it. On a somewhat larger scale, deserts may expand, cutting off populations that cannot live under desert conditions. Allopatric speciation is the most common form of speciation. It is distinguished from reproductive isolation mechanisms because it is not part of the species' biology. The divergence of populations into new species is known as divergent evolution.

Physical barriers that can separate a subpopulation from its original population species can include:

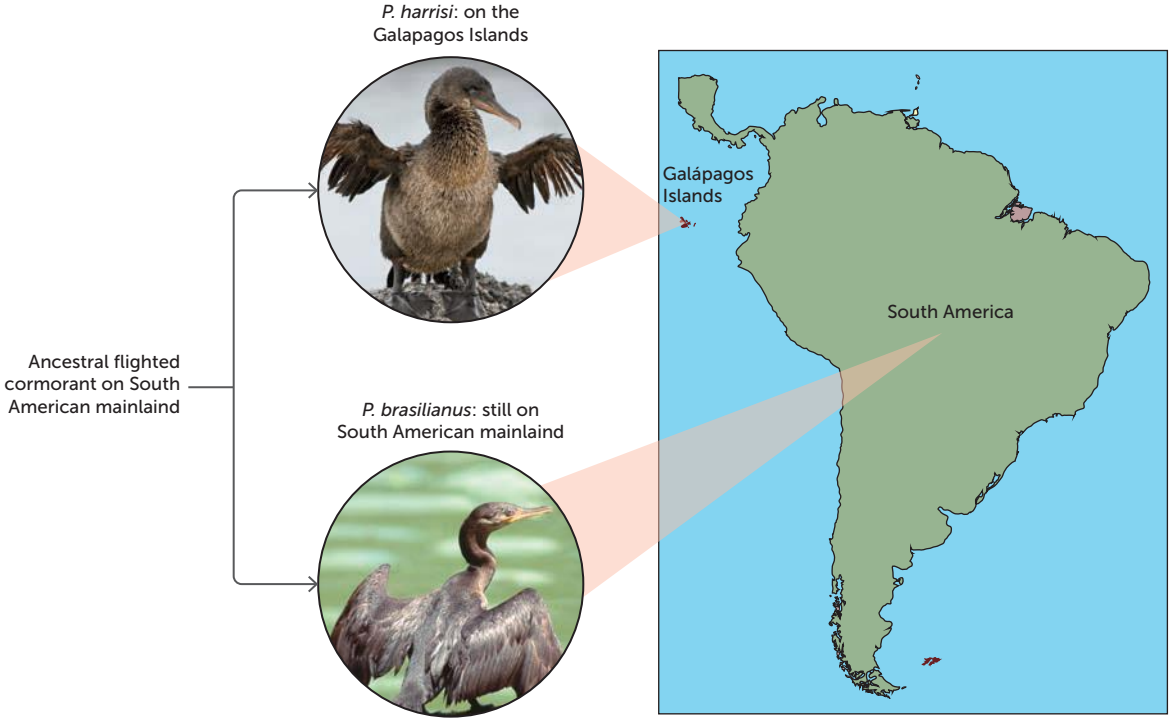
- water, for terrestrial organisms
- land, for aquatic organisms
- mountains.

New physical barriers can arise due, among other things, to:

- continental drift
- rising sea levels
- climate change.

Islands are home to many examples of allopatric speciation. On the Galápagos Islands, Darwin noticed a flightless cormorant. This species most likely originated from a small population of ancestral flying species that reached the islands from the South American mainland. The two populations would have been physically isolated by the 1000 km of ocean between the islands and the South American mainland. There would have been no gene flow between the two populations. The islands were totally free from predators. The reduced predation changed the selection pressures acting on this cormorant population. There were still selection pressures for efficient movement underwater, but there was less pressure for efficient flight. This led to a reduction in the size of the wings in the cormorant population, to a morphology that was well suited to movement under water but which no longer allowed flight.

The flightless cormorant (*Phalacrocorax harrisi*) of the Galápagos Islands diverged from flighted cormorants on the mainland through allopatric speciation. *P. harrisi* is most closely related to cormorants such as the neotropic cormorant (*P. brasilianus*), which is widespread throughout tropical regions of South and North America (Figure 9.24). Phylogenetic studies have only recently identified the mainland species (all flighted) to which the Galápagos cormorant is most closely related.



FLPA/Krystyna Szulecka (top); Getty Images/Kevin Schafer (bottom)

FIGURE 9.24 *Phalacrocorax harrisi* and *P. brasilianus* demonstrate allopatric speciation.

The more recent arrival of feral dogs and cats to the islands has once again led to a change in selection pressures on this animal. There have been dramatic reductions in the cormorant population, which is not well adapted to the new predation pressures because it cannot fly. It is now recognised as an endangered species.

Key concept

Most speciation seems to occur as a result of populations becoming physically separated through geographical isolation, leading to disruption of the gene flow. This process is called allopatric speciation.

TABLE 9.4 The process of allopatric speciation

STEPS	VISUAL AID
1 Subpopulations A parent population divides into two or more subpopulations.	A parent population of a single species is distributed over a broad range, resulting in subpopulations.
2 Isolation by a physical barrier A physical barrier such as a mountain range, river or desert separates and isolates the subpopulations.	
3 No gene flow Due to the physical barrier, the two subpopulations are genetically isolated. There is no migration between the two subpopulations.	
4 Different selection pressures The different environments apply different selection pressures. The two subpopulations evolve independently from each other.	A physical barrier such as a higher sea level separates two populations and prevents gene flow. Populations adapt to differing environments on opposite sides of the barrier (natural selection). Genetic drift causes different random changes in both subpopulations.
5 Natural selection For each subpopulation, different advantageous alleles are selected for the survival of the fittest, resulting in different allele frequencies over generations.	
6 Genetic drift Genetic drift occurs independently in the subpopulations, causing different alleles to be passed to offspring randomly.	
7 Two different species Small micro-evolutionary genetic differences over generations accumulate to become large differences, until the two groups become two different species, no longer able to interbreed to produce viable, fertile offspring. They are then reproductively isolated.	Two new species. If the barrier is removed, the populations may recolonise the intervening area and mingle, but do not interbreed.

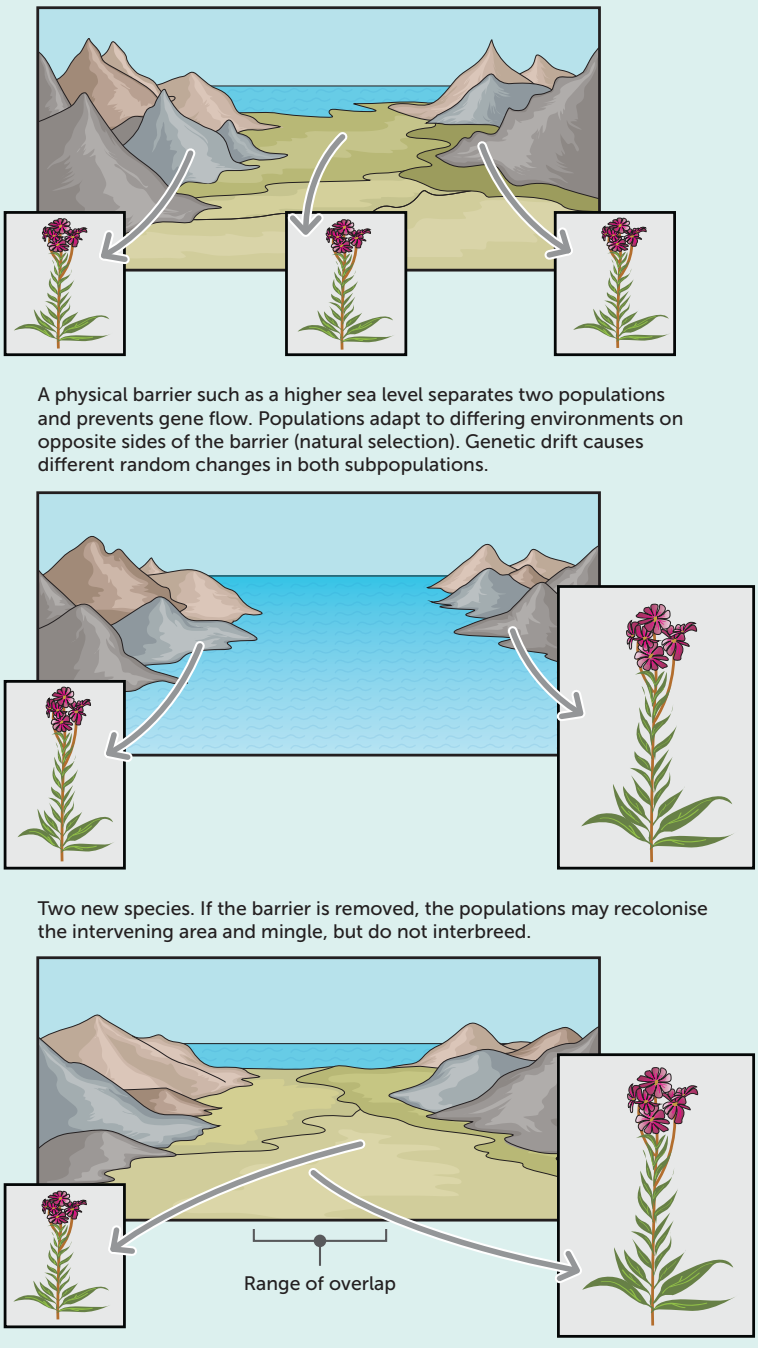


FIGURE 9.25 The process of allopatric speciation

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Sympatric speciation

Allopatric speciation seems to have been the main mechanism producing new species throughout evolutionary history. But sometimes species diverge without any obvious physical or geographical isolation. **Sympatric speciation** refers to the evolution of two or more new species from a single population within the same place.

How can new species arise without physical separation? It might be that groups within a single population feed on different things, or choose mates based on different characteristics. They may also choose to mate at different times. Genetic separation may occur due to the various pre-zygotic and post-zygotic processes introduced on pages 312 and 313, respectively. There are not as many clear examples of this type of speciation, but a few are quite striking, as in the case of *Magicicada* (Figure 9.23, page 312).

Question set 9.7

REMEMBERING

1. Describe the different types of selection that can lead to speciation.
2. Describe the mechanisms that can lead to the isolation of populations.
3. Explain how isolation of populations can lead to micro-evolutionary changes.
4. Explain 'allopatric speciation' and provide an example.
5. What factors can act as geographic barriers?

UNDERSTANDING

6. What effects can isolating mechanisms have on a population?

9.8 EXTINCTION OF SPECIES

The fossil record shows that nearly all the species that ever lived are now extinct. In most cases, they represented the end of an evolutionary lineage and left no descendants. Although extinction occurs quite regularly, there have been periods when the rate of extinction has been very high. These are referred to as **mass extinctions**.

The most dramatic mass extinction event, often called the 'Great Dying' appears to have occurred at the end of the Permian period 250 mya. This appears to have coincided with one of the most extensive periods of volcanic activity Earth has ever seen. In fact, some scientists believe that this event went close to wiping life out completely. One of the few survivors of this catastrophe, however, was the ancestor of the dinosaurs, one of the most successful vertebrate groups ever to have evolved.

Australian bushfires push threatened species towards extinction

Over 1 billion animals died during the 2019/2020 Australian bushfire season. During this devastating season, bushfires burned with significantly higher intensity compared with previous seasons due to high temperatures and drought. Endangered species, including the long-footed potoroo, Kangaroo Island's glossy black-cockatoo and Batemans Bay's spring midge orchid were pushed towards extinction. For some species, their entire distribution area was burned. In addition to the immediate mortalities from the fire, there would be ongoing mortalities after the fire from starvation, lack of shelter, and attacks from predators such as foxes and cats, which are attracted to fire-affected areas to hunt. Large parts of Kangaroo Island are designated as conservation areas to protect animals such as sea lions, penguins, kangaroos, koalas, bees, Kangaroo Island dunnarts and various birds, including glossy black-cockatoos. Many members of these species were lost to the fire. The devastating wildfires undid decades of careful conservation work on Kangaroo Island and have threatened to wipe out some of the island's unique fauna altogether.

The Australian Academy of Science press release
Watch this video to further your understanding of the effects of the 2019/2020 bushfire season in Australia

An ecologist at the Australian National University, Professor Sarah Legge, argued that the driver of so much land being burned at once with such intensity was climate change. The extended drought and high temperatures experienced over much of Australia are unprecedented.

Animals able to escape fires survive, and those who can't (particularly slow-moving, land-dwelling animals) perish. This will impact on the evolution of species in Australia. Regeneration and evolution by natural selection is slow; extinction can be fast. This means biodiversity is lost.

The National Aeronautics and Space Administration (NASA) estimated that thousands of koalas have died, possibly tens of thousands, just on Kangaroo Island alone. Koala populations that survive the fires are likely to be cut off from one another, lowering their genetic diversity and threatening their long-term survival. Sudden reductions in population size can cause genetic bottlenecks that lead to inbreeding, which can reduce reproductive fitness and make extinction more probable. To help the surviving koala populations, many volunteers will be needed for tree planting and to help with emergency care and conservation work.

The Australian Academy of Science recommends that in order to reduce the worst impacts of climate change, we need ensure global warming does not exceed 1.5°C above the long-term average. The underlying reasons for these unprecedented bushfires need to be addressed to enable the long-term survival of koalas, and other animals and plants.

Ryan Pollock Photography

FIGURE 9.26 This joey was rescued and treated for burns after the Stirling Ranges bushfires of 2019/2020.

Key concept

Extinctions have occurred naturally over geologic time. The recent Australian bushfires devastated populations of native animals, pushing many already endangered species closer to extinction.

Animals killed in bushfires
Click on the video series and watch 'At least a billion animals killed in bushfires'.

Preventing extinction by preserving genetic diversity

Populations with reduced diversity face increased risk of extinction, so conservation projects usually focus on maintaining genetic diversity. When large-scale extinctions occur, not all species are lost. Some seem to be more at risk than others. Rapid extinction events can lead to greater loss of large organisms than of small ones. A large distribution area is generally a big advantage, because it may allow some pockets of habitat to survive. Large population size can also be some protection, because the population is likely to have a more diverse gene pool and thus a greater variety of alleles and phenotype options as the pressures from natural selection change.

Question set 9.8

REMEMBERING

1. Define extinction.
2. Define mass extinction. When was the largest mass extinction event on Earth?

UNDERSTANDING

3. Why do many scientists believe the 2019/2020 Australian bushfires were the result of climate change?
4. Why does the Australian Academy of Science recommend that an increase in global warming must be limited to less than 1.5°C?

Getty Images/Lisa Maree Williams

FIGURE 9.27 A rescued koala injured in the 2019/2020 bushfire on Kangaroo Island, South Australia

SCIENTIFIC LITERACY

WA conservation, extinction and evolution

Over the past 100 years, more mammals have become extinct in Australia than anywhere else in the world. In WA, since European colonisation:

- 12 mammal species have become extinct
- 7 mammal species have disappeared from the mainland, but remain on a few offshore islands
- more than 30 mammal species have declined significantly or are threatened with extinction.

Western Shield is the flagship wildlife recovery program of the Government of WA's Department of Biodiversity, Conservation and Attractions, and the Parks and Wildlife Service. It was launched in 1996 and is now one of the biggest wildlife conservation programs ever undertaken in Australia. The aim of the project is to recover native animal populations in the wild, through control of foxes and feral cats, and by reintroducing native animals to their former habitats.

Department of Biodiversity, Conservation and Attractions - Western Australia

FIGURE 9.28 WA's Western Shield recovery program

Western Shield aims to return the balance of native animals in selected areas of WA's environment to levels comparable with pre-European colonisation. It has a particular focus on threatened species.

Western Shield suggests viewing the 53-minute video 'Before it's too late: Australia's Mini Marsupials'. It tells of the impact of European colonists on the endemic species and on the Indigenous peoples, and shows people being trained to become wildlife managers.

Australia's mini marsupials
Learn about the impact of European colonialists.

The most famous species to have become extinct is the Tasmanian tiger. The video warns that the woylie, numbat and honey possum are also close to extinction.

Woylies are an example of a species unable to adapt to the drastic changes in their environment. The most significant changes to their environment include the introduction of species such as feral cats, and habitat loss due to land clearing and climate change. Woylie populations have declined from 225 000 to around 10 000–20 000 in the last 15 years. They once inhabited more than 60% of mainland Australia, ranging through WA, the Northern Territory, South Australia, Victoria and New South Wales. Now, they can only be found in small pockets in WA and on offshore islands in South Australia.

Murdoch University led a study on the genetic diversity loss of woylies, which was published in 2015. They found, when compared with ancient DNA, that woylies had lost a significant amount of genetic diversity, and recommended assisted migration (and thus assisted gene flow) to increase their reproductive fitness.

Honey possums are the only marsupial in the world that feeds solely on nectar and pollen. A deadly plant disease known as phytophthora is partly responsible for the decline in honey possum numbers. Phytophthora kills the trees on which the possum feeds.

Honey possums play a significant pollinating role in their ecosystem. In recent years, too-frequent fires have clearly disrupted their population growth and genetic diversity. Don Bradshaw, a retired Professor of the University of Western Australia, campaigned for the protection and conservation of the honey possum and other endangered species.

Alamy Stock Photo/Nature Picture Library

FIGURE 9.29 Honey possums are threatened by habitat loss.

Honey possums are only found in south-western Australia. They have a very limited diet – the sweet nectar from plants such as banksias and bottlebrushes. One adaption they have developed is a long snout to reach the nectar, and newborn baby honey possums weigh only about 5 mg.

The spread of the disease phytophthora, caused by *Phytophthora cinnamomi* (dieback), throughout southern WA has severely impacted *Banksia ilicifolia* trees, which are the honey possums' primary food source, and the impact has been greater in burnt areas than in unburnt areas. Professor Bradshaw conducted a study on honey possum recovery after two fires. Analysis of catch-per-unit-effort and population density of honey possums over the whole 29-year period of the study showed that numbers had not declined in the long-unburnt southern area of the study site, despite the spread of dieback and loss of banksia trees. Recovery numbers were less encouraging in the burnt areas. Given predictions of increasing fire frequencies due to climate change and the increased use of prescribed burning to protect human life and property, it is imperative that areas harbouring honey possums be protected from too-frequent fire if this iconic species is to persist. It is close to extinction.

Department of Biodiversity, Conservation and Attractions - Western Australia

Questions

1. Name a new factor that entered the environment of the woylie with European colonisation.
2. Describe how feral cats and foxes have introduced additional natural selection within populations of small and medium-sized mammals endemic to WA.
3. Honey possums are an endangered species. Why should we care that they are close to extinction?

Too little, too late for the Leadbeater's possum?

CASE STUDY

Professor David Lindenmayer is one of Australia's leading landscape ecologists and conservation biologists. Based at the Australian National University in Canberra, his research specialises in areas such as forest ecology and management, and habitat fragmentation. He is the leading expert on the Leadbeater's possum. In an interview on the ABC radio program *PM* in August 2013, he described how his research suggests future strategies for the conservation of the Leadbeater's possum.

In the 1950s, the Leadbeater's possum was known only from a few specimens collected in the early 1900s. It was declared extinct, but a small population was rediscovered in 1961. The isolated population grew to 7500 individuals in the 1980s. Then, bushfire in 2009 nearly wiped out all of their habitat and numbers. Down to less than an estimated 1000 individuals, this species is currently experiencing a genetic bottleneck and is threatened with extinction. Professor Lindenmayer has been studying the Leadbeater's possum for more than 30 years, in an attempt to develop an appropriate conservation plan.

Population modelling analysis has predicted that Leadbeater's possum populations of below 50 are unviable, whereas a population greater than 200 could survive 100 years. He used metapopulation analysis to assess the effect of habitat patch size, connectivity between patches, fire and logging on the species' survival. Sustainable populations need patches of more than 50 hectares of mountain ash forest that is older than 120 years, with 6–12 tree hollows per 3 hectares. As mountain ash trees take decades to regenerate after fire or logging, a 10-year study trialled the provision of

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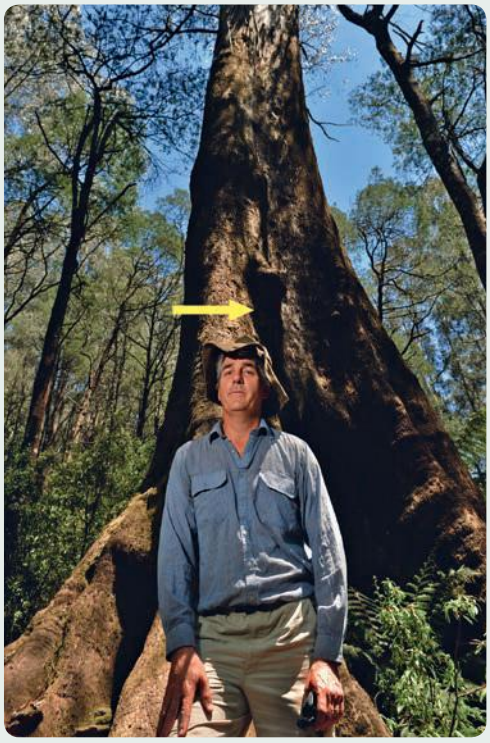
nest boxes in Leadbeater habitat. Unfortunately, the artificial hollows were not used. In addition, the research has shown that logging significantly degrades habitat for Leadbeater's possum and renders it unsuitable for 150 to 200 years. The science is now 30 years in the making and it's very strong. The path forward is clear. If we don't log strategically, Leadbeater's possum is going to go extinct.

Questions

1. Outline the main habitat features essential for the survival of the Leadbeater's possum's remaining population.
2. Apply your understanding of selection pressures and describe those that are likely to be acting on the Leadbeater's possum.
3. Fossil evidence shows the historical distribution of the Leadbeater's possum included an area in New South Wales. Suggest how this species may have evolved and how its distribution may have become so limited and fragmented.



Fairfax Photos/ Michael Clayton-Jones

FIGURE 9.30 Professor David Lindenmayer in a Victorian mountain ash forest. A possum hole is shown in the trunk of the tree (yellow arrow).

Auscape. All Rights reserved/Jean-Paul Ferrero

FIGURE 9.31 Leadbeater's possum (*Gymnobelideus leadbeateri*) is a small possum currently restricted to the small remaining pockets of old-growth mountain ash forests in the Central Highlands of Victoria. It is an endangered species.

CHAPTER 9 INVESTIGATION

Developed by Southern Biological

Natural selection with brine shrimp

9.1 INVESTIGATION

Background

Natural selection was proposed by Charles Darwin to explain how new species evolve. All types of living things have small differences between the individuals in the species. If one of those differences allows the individual to live longer, they will likely have more offspring. As that trait is passed on, the population starts to look more like the successful individual. Over time, the species changes.

Aim

To explore how hatching viability of *Artemia* sp. is impacted by different saline level environments.

Time requirement

45 minutes

Materials

- Brine shrimp eggs (cysts)
- 4 Petri dishes
- 0.5%, 1.0% and 2.0% solutions of salt water
- 1 fine brush
- 4 microscope slides
- 4 strips of double-sided tape
- 1 stereo microscope
- 1 permanent marker
- Distilled water
- 1 graduated cylinder
- Light bank or source of light

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Brine shrimp may cause an allergic reaction in some people.	Ensure appropriate personal protective equipment is used at all times and be mindful of any known allergies.

Procedure – Preparing eggs for hatching (day 1)

1. Using a permanent marker, label 4 Petri dishes: 0%, 0.5%, 1.0%, 2.0%.
2. Form your hypothesis (e.g. the cysts will have the greatest hatching viability in the solution, because brine shrimp thrive in high saline environments).
3. Using a graduated cylinder, measure 30 mL of each saline solution and pour it into the appropriately labelled Petri dish.
4. Collect 4 microscope slides. Measure and cut a 1.5 cm strip of double-sided tape and gently adhere it to a microscope slide. Repeat this step for the remaining microscope slides.
5. Lightly touch the fine brush to the side of the container of brine shrimp eggs. Collect approximately 20–30 eggs on the brush. Do not collect too many eggs as you will be required to count each one.
6. To adhere the eggs to the double-sided tape, lightly press the brush onto the tape on the microscope slide. Repeat this step for the remaining 3 microscope slides.

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7 Using a microscope, count the number of eggs on the first slide. Copy Table 9.5 and use it to record your information.
8 Once the eggs have been counted, place this slide into the 0% salt solution Petri dish. Place the slide with the tape side facing up.
9 Count the eggs on each slide and place them in the respective salt solution. Record the egg count in Table 9.5.
10 Place the Petri dishes under a light bank and allow them to rest at room temperature for 24 hours.

Procedure – Data collection (days 2 & 3)

11 Using a stereo microscope, examine the contents of each Petri dish.
12 By this stage, you should see some brine shrimp have hatched and are swimming in the salt solution. Record this information in Table 9.5.
13 Calculate the hatching viability of each dish at 48 hours by dividing the number of shrimp swimming by the initial number of eggs in the Petri dish. Repeat this process for all 4 Petri dishes and record the results in Table 9.5. Copy Table 9.6, round up your calculations to the nearest hundredth, and add your information to the class data in Table 9.6.
14 Calculate the mean. Round your answers to the nearest hundredth and add them to Table 9.6.

Results

TABLE 9.5 Group data for hatching viability of brine shrimp in varying levels of salinity

% NaCl	0 HOURS	24 HOURS			48 HOURS			
	NO. OF EGGS	NO. OF EGGS	NO. DEAD OR PARTIALLY HATCHED	NO. SWIMMING	NO. OF EGGS	NO. DEAD OR PARTIALLY HATCHED	NO. SWIMMING	HATCHING VIABILITY (%)
0%								
0.5%								
1%								
2%								

TABLE 9.6 Class data for hatching viability of brine shrimp in varying levels of salinity

GROUP	SALINITY			
	2%	1%	0.5%	0%
1				
2				
3				
4				
5				
6				
7				
8				
Mean hatching viability				

Draw a graph of your results, ensuring you include all labels and units.

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Discussion

1. Describe two conditions that were controlled in this experiment.
2. Which Petri dish had the highest hatching viability? Which had the lowest? Suggest possible reasons for these results.
3. Was your hypothesis correct? Why or why not?
4. Based on your individual and class data, is there enough evidence to conclude that environments of different salinities affect the hatching viability of brine shrimp?

Taking it further

What other conditions may impact the hatching viability of brine shrimp? Design an experiment to investigate another environmental factor that may impact hatching viability.

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WS
Chapter 9 Activity sheet

CHAPTER 9 SUMMARY

- The main mechanisms of evolution are mutation, natural selection, genetic drift, and gene flow (through migration).
- New alleles arise through mutations, which are a key source of variation within a population.
- Natural selection acts on variation in the phenotype of a population. This variation is inheritable.
- The modern theory of evolution takes into account all that we now understand of how our traits are inherited and is called the modern synthesis.
- A population is a group of individuals of the same species that live in the same geographic area and interbreed, producing fertile offspring. The sum total of all alleles present in a population is called the gene pool.
- Selective breeding (artificial selection) is the selection of animals and plants with desirable characteristics for breeding, resulting in changes in allele frequencies in gene pools over time.
- Sexual selection occurs when individuals with certain inherited characteristics are more successful than other individuals in finding mates.
- The bottleneck effect refers to genetic drift that occurs when there is a significant reduction in population size, resulting in genetic diversity that is non-representative of the original population's allele proportions. This can lead to a decrease in the gene pool of a species.
- The founder effect is observed when a new population is established by a small number of individuals, leading to a population with limited genetic diversity.
- Gene flow is the movement of genes between different populations.
- A gradual change in the gene pool of a population is called micro-evolution.
- Speciation and macro-evolutionary changes result from an accumulation of micro-evolutionary changes over time.
- Migration is the movement of individuals of a population into or out of a region. Migration can result in gene flow.
- The formation of new species often involves reproductive isolation combined with selection pressures, leading to a disruption of the flow of genes.
- The splitting of a single species into multiple new species usually involves physical or geographical isolation. This is allopatric speciation.
- Speciation always involves significant changes in the gene pool.
- Major climatic and geological changes have led to the evolution of multiple new species from a single group, a process referred to as adaptive radiation.
- Severe changes in climate and other physical conditions have sometimes led to the extinction of many species, an event referred to as a mass extinction.
- Populations with reduced genetic diversity face increased risk of extinction.

CHAPTER 9 GLOSSARY

Accumulation The process of alleles or traits gradually becoming more common over generations

Adaptive evolution Changes in a population of organisms that make that population better adapted to its environment over time

Adaptive radiation The process by which a species rapidly diversifies into many taxa with differing adaptations; it can be triggered by many factors, such as the emergence of reproductive barriers within a population, changes in the availability of resources, new challenges or new opportunities; it is a type of divergent evolution

Allopatric speciation Speciation that occurs due to physical or geographic isolation

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Artificial selection (selective breeding) The intentional breeding or reproduction by humans of individuals with desirable traits, resulting in changes in allele frequencies in gene pools over time; the traits are beneficial to humans

Biological fitness The capacity of an individual to survive and produce viable, fertile offspring

Biological species concept A definition of species based on whether members can interbreed to produce fertile offspring

Bottleneck effect A random reduction in the size of a population, which can lead to a reduction in the gene pool because of the misrepresented allele proportions; it can occur when a catastrophic event or a period of adverse conditions drastically reduces the size of a population

Descent with modification Darwin's terminology for the way life today has descended and evolved from common ancestors that were generally different from their modern descendants

Divergent evolution A process whereby related species evolve new traits over time after living in different habitats, becoming increasingly different from their common ancestor and from one another, giving rise to new species

Evolution The process of cumulative, gradual, heritable change in a population of organisms that occurs over many generations and a relatively long time

Founder effect A random reduction in a population that occurs when a few individuals become isolated from a larger population and form a new population that does not carry all the alleles that were present in the original population

Gene flow The transfer of alleles that results from emigration, immigration and migration of individuals between populations

Gene pool A collection of all the alleles for all the genes in the reproducing members of a population at a given time; it is the genetic reservoir from which a population can obtain its traits

Genetic drift A change in the gene pool of a population as a result of chance; it usually occurs more noticeably in small populations

Hybrid offspring Offspring from parents of two different species

Inheritable Capable of being passed on to the next generation

Isolating mechanism A mechanism that prevents organisms from mating or producing viable offspring

Macro-evolution The evolution of new groups of organisms comprising many related species through multiple speciation events; includes adaptive radiations

Mass extinction Extinction of many species over a relatively short (geological) period of time

Micro-evolution Change in the gene pool below species level; any small-scale change in the gene pool of a population

Modern synthesis The theory of evolution incorporating our current understanding of how traits are inherited

Morphological species concept Definition of a species using measurable anatomical criteria and characteristics

Mutation A permanent change in the DNA sequence of a gene; a source of new alleles in a population's gene pool; the process of generating a mutation

Natural selection Occurs when selection pressures in the environment confer a selective advantage on a specific phenotype that enhances its survival and reproduction. It is a process in which individuals that have certain inherited traits are more likely to survive and reproduce at higher rates than other individuals because of those traits. It can cause changes in a population's allele frequencies (gene pool) and therefore is a mechanism for evolution

Population A group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

Post-reproductive isolating mechanism A mechanism that prevents fertilisation occurring or an embryo developing into viable offspring if fertilisation does occur

Pre-reproductive isolating mechanism A mechanism that prevents organisms from being able to interact to reproduce

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Reproductive isolation When a single population becomes two separate populations that are unable to interbreed due to physical, biological or behavioural barriers

Selection pressure A factor that influences the survival of an individual within a population

Sexual dimorphism Different morphologies (often in shape or size) between males and females of a species

Sexual selection A selection process that occurs between males or between females in a population for an inherited trait that assists in copulation or the winning of a mate

Speciation The evolution of one or more new species from an ancestral species. A population is considered a new species when it can no longer interbreed with the ancestral species.

Species A group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same

Sympatric speciation Speciation that occurs without physical or geographic isolation

Theory of evolution States that all organisms have developed from previous organisms and that all living things have a common ancestor in some initial form of primitive life. It also states that all organisms are fundamentally similar because their basic chemistry was inherited from this very first organism.

Variable trait A trait that varies in the population due to differences in alleles carried by different individuals

Variation The diversity of genetic and phenotypic traits within and between populations

CHAPTER 9 REVIEW QUESTIONS

Remembering

1 Define:
   - a gene pool
   - b allele frequency
   - c genetic drift.

2 Draw a diagram to illustrate the founder effect.

Understanding

3 Explain the term 'bottleneck' and what effect a bottleneck may have had on the human gene pool.

4 Draw a diagram to summarise the ways natural selection has occurred among the peppered moth of Great Britain, as described in this chapter.

5 Provide an example of how an understanding of changing gene pools is important in understanding evolutionary change.

Applying

6 Apply the definition of micro-evolution to a discussion of whether modern humans are still evolving.

7 Herbert Spencer used the phrase 'survival of the fittest' to describe Darwin's concept of natural selection. Outline the ways in which this term could be misleading.

8 In the 2019/2020 bushfire season, many areas of Australian bush were burnt, leaving fragments of habitat. A group of greater gliders (Figure 9.32) were cut off from the main remnant of their population due to lost habitat and they could no longer mate with them.
   - a Assuming there was a sufficient gene pool in the subpopulation for them to survive, describe how this could lead to speciation.
   - b Would this be allopatric speciation? Explain.

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Analysing

9 Construct a genetic drift diagram that illustrates how a trait can become extinct over a few generations.

10 Identify the key difference between Darwin's original conception of adaptive evolution through natural selection and what is referred to as the 'modern synthesis of the theory of evolution'.

11 Explain why processes such as genetic drift, the founder effect and sexual selection are not regarded as examples of adaptive evolution.

12 When a mutation occurs in a large population, it has very little effect on the population as a whole. Even so, mutations are vital to the process of evolutionary change. Explain why.

13 Over the last 30 years, many new pre-human fossils have been found, but scientists often find it difficult to agree on whether or not they should be classified as new species. Account for this difficulty in terms of our current understanding of the species concept.

Alamy Stock Photo/Auscape International Pty Ltd

FIGURE 9.32 A greater glider

14 To establish the extent of relatedness between species, several techniques have proved to be useful. One of these measures the difference between the DNA of various species. When the DNA of the orangutan, gorilla, chimpanzee and humans were compared, it was found that less than 1% of the total DNA of these species differed. A rapidly evolving region of the genome was analysed. This region had less than 3.5% variation, as shown in Table 9.7.

TABLE 9.7 Percentage divergence of nucleotide sequences in a rapidly evolving section of nuclear DNA in four primate species

		COMPARED WITH DNA FROM ...			
		HUMAN	CHIMPANZEE	GORILLA	ORANGUTAN
DNA FROM ...	HUMAN	–	1.56	1.69	3.30
	CHIMPANZEE	1.56	–	1.82	3.42
	GORILLA	1.69	1.82	–	3.39
	ORANGUTAN	3.30	3.42	3.39	–

a From the DNA data comparison, which primate(s) seem to be most closely related to humans?

b Of the non-human primates, which seem to be most closely related to one another from this data?

c From the data, which pair of primates do you think shared the most recent common ancestor?

d Based on the data, construct a possible phylogenetic tree.

Evaluating

15 The long-billed black-cockatoo (*Calyptorhynchus baudinii*) is found in the south-west of WA. It lives in the tall jarrah–karri forests and eats the seed capsules of eucalypts. Very similar birds, known as short-billed black-cockatoos, live in the inner wheat belt around Geraldton and

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generally avoid forests. They eat pine seeds as well as hakea and banksia seeds. Initially the short-billed black-cockatoos were classified as a subspecies. Following further investigation, over time, the subspecies was reclassified as *Calyptorhynchus latirostris*, a separate species. Bill length is described as an adaptation to the different environments that the birds occupy.

a What is meant by the term adaptation?
b What is meant by the term subspecies?
c Using the concepts you have learned in this chapter, outline the steps that might have taken place for the two subspecies to become two separate species.

Creating

16 Design a diagram that clearly summarises the various mechanisms leading to evolutionary change. Your table or diagram should indicate which of these changes lead to populations becoming better adapted to a change in their environment.

PRACTICE EXAM QUESTIONS

1 Select the correct statement.
A Natural selection can result in the evolution of new species, but artificial selection cannot.
B Artificial selection can result in the evolution of new species, but natural selection cannot.
C Both natural and artificial selection can result in the evolution of new species.
D Neither natural nor artificial selection can result in the evolution of new species.

[Q6 2019 SCSA]

2 Genetic differences between populations are reduced by:
A gene flow
B mutation
C sexual selection
D genetic drift.

[Q26 2019 SCSA]

3 A population of humpback whales was hunted almost to extinction before hunting was stopped. Since then, the population has been increasing. The genetic diversity of the population will:
A not have been affected by the changes in population size
B not recover as quickly as the population size
C have returned to pre-hunting levels as soon as the hunting stopped
D be decreasing as the population size increases.

4 Which of the following situations is most likely to lead to allopatric speciation?
A Members of a fruit fly population breed on different species of trees.
B Members of a spider population are separated by a housing development.
C Members of a fish population migrate to a new area to breed.
D Members of a plant population tolerate different quantities of heavy metals.

[Q9 2018 SCSA]

5 The males of some species of beetle have much larger horns than the females. These horns decrease the chances of the males surviving, but increase their chances of finding mates. The large horns of the male beetles are likely to have evolved via:
A natural selection, in which the males compete with one another for mates
B natural selection, in which the females compete with one another for mates
C sexual selection, in which the males compete with one another for mates
D sexual selection, in which the females compete with one another for mates.

[Q27 2018 SCSA]

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6 The woolly mammoth was a large mammal that became extinct approximately 4000 years ago. Reduced genetic diversity associated with a small population size contributed to the extinction of this species. The extinction was probably due to:

- **A** artificial selection
- **B** natural selection
- **C** mutation
- **D** genetic drift.

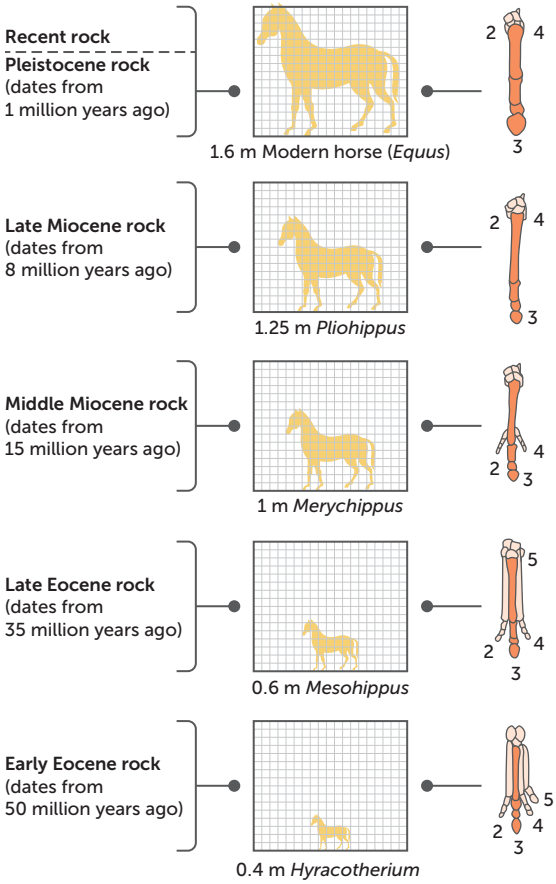
[Q3 2017 SCSA]

7 Dung beetles are a type of insect and feed on animal faeces. A recent survey identified over 500 species of dung beetle that are native to Australia.

- **a** Define a species. (2 marks)
- **b** Explain how new species of dung beetle could evolve by allopatric speciation. (5 marks)
- **c** Most species of dung beetle are small and experience genetic drift. Describe how genetic drift affects the genetic composition of populations. (3 marks)
- **d** One species of dung beetle has males with larger horns than the females. The larger horns make movement and eating more difficult. Explain how the larger horns in the males of this species could have evolved. (5 marks)

[Q31a–d 2019 SCSA]

8 The following diagram shows the evolution of height and forefeet in modern horses and their extinct ancestors over the past 50 million years. The digits ('fingers') of the forefeet are labelled 2 to 5.



Is the evolution of horse forefeet an example of micro-evolution or macro-evolution? Explain your answer. (4 marks)

[Q32e 2018 SCSA]

9 Explain how speciation and macroevolutionary changes result from an accumulation of micro-evolutionary changes over time. (10 marks)

[Q36b 2019 SCSA]

10 Describe how micro-evolution could result in the development of salt tolerance in a species of plant. (10 marks)

[Q37a 2019 SCSA]

11 Discuss how genetic drift and gene flow change allele frequencies in the gene pool of a population. (10 marks)

[Q36b 2018 SCSA]

12 Describe the process of allopatric speciation. (10 marks)

[Q37a 2017 SCSA]

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UNIT 4

SURVIVING IN A CHANGING ENVIRONMENT

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10 HOMEOSTASIS AND THERMOREGULATION

Getty Images/Max shen

CHAPTER 10 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Homeostasis is required in order to maintain certain internal factors within a tolerance range. Can you identify the parts of an animal's body that can detect changes in the internal and external environments?
2 Can you list factors inside a mammal's body that require maintenance within a set range for that mammal to survive?
3 What processes occur inside your body to assist in maintaining your body temperature at 37°C when you move from an inside air-conditioned temperature of 25°C to an outside temperature of 40°C?

SCIENCE UNDERSTANDING

» homeostasis is the process by which the body maintains a relatively constant internal environment; it involves a stimulus–response model in which change in external or internal environmental conditions is detected and appropriate responses occur via negative feedback
» changes in an organism's metabolic activity, in addition to structural features and changes in physiological processes and behaviour, enable the organism to maintain its internal environment within tolerance limits (temperature, nitrogenous waste, water, salts, and gases)
» thermoregulatory mechanisms include structural features, behavioural responses and physiological mechanisms to control heat exchange and metabolic activity; animals can be endothermic or ectothermic

SCIENCE INQUIRY SKILLS

» conduct investigations, including using models of homeostasis and disease transmission, safely, competently and methodically for valid and reliable collection of data

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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10.1 HOMEOSTASIS

Homeostasis is defined as the processes involved in maintaining a constant internal environment, within tolerance limits, despite changes in the internal and external environment. Homeostatic regulation occurs in two stages. Stage one is the detection of a change from the stable state, known as the **stimulus** (plural **stimuli**). Stage two is the **response** to the stimulus, which can be described as counteracting the change. Therefore, to understand homeostatic regulation, a **stimulus–response model** can be used. The purpose of homeostatic regulation is to maintain internal factors around a set normal value. When the factors deviate away from the value, homeostatic **adaptations** will attempt to bring the factor back to the normal value.

Homeostasis Part 1
Watch this video for an introduction to homeostasis.

Homeostasis Part 2
Learn more about homeostasis.

Living organisms carry out a series of chemical reactions in order to continue living. A linked series of reactions is known as a biochemical pathway. The sum total of these chemical reactions is called **metabolism**. The metabolic reactions are controlled by **enzymes**. Without enzymes, the chemical reactions would proceed too slowly to keep the organism alive. Enzymes are reusable, biological catalysts that lower the activation energy of chemical reactions, enabling them to proceed faster. They are proteins that are sensitive to factors such as temperature and **pH**. Enzymes have certain tolerance limits within which they can avoid denaturation and function effectively. When an enzyme is denatured, it changes shape and becomes useless. Protein shape is influenced by salinity, pH, temperature and other environmental factors. Homeostatic processes maintain all those factors within ranges favourable for the operation of enzymes.

Homeostatic regulation is required in all organisms in order to maintain the correct environment for metabolic reactions. It is a necessity for survival. Just as there are a huge variety of organisms in our world, there are huge variations in the level of homeostatic regulation required by different organisms. Some organisms regulate many internal factors in widely varying environments, while others do little more than contain their internal chemicals as well as they can in fairly constant environments. Homeostasis (steady state) means the environment inside living organisms stays the same, regardless of external environmental changes. Organisms have evolved to be able to achieve this when the changes are not too extreme. When the changes are extreme, the internal environment of an organism may be driven outside tolerance limits, causing illness or death. For example, on a mountain top, the human body can produce extra red blood cells to keep the level of oxygen in the body from getting too low, even though the level of oxygen outside is lower than at sea level. However, at a certain point there's just not enough oxygen outside and the body cannot cope.

This chapter explores the stimulus–response model of homeostasis, **negative feedback**, and the **structural features**, **behavioural adaptations** and **physiological processes** that help organisms to maintain a relatively constant internal state – a state of homeostasis – and survive in their environments.

Detecting the stimulus

Signals may come from the external environment, from other parts of the organism or from within the cells. Stimuli may be physical (e.g. light, heat, pressure) or chemical (e.g. hormones, neurotransmitters). There are millions of external and internal **receptors** that allow an organism to respond to stimuli. The five main types of receptors are: **chemoreceptors**, **mechanoreceptors**, **photoreceptors**, **thermoreceptors** and **pain receptors**.

Internal receptors receive signals from within the body about the internal environment. Examples of internal signals are an increase in carbon dioxide concentration or low pH levels. The **interstitial fluid** (the fluid that bathes the cells) and the blood plasma are the medium of the internal environment. Cells exchange substances with the interstitial fluid across membranes (Figure 10.1).

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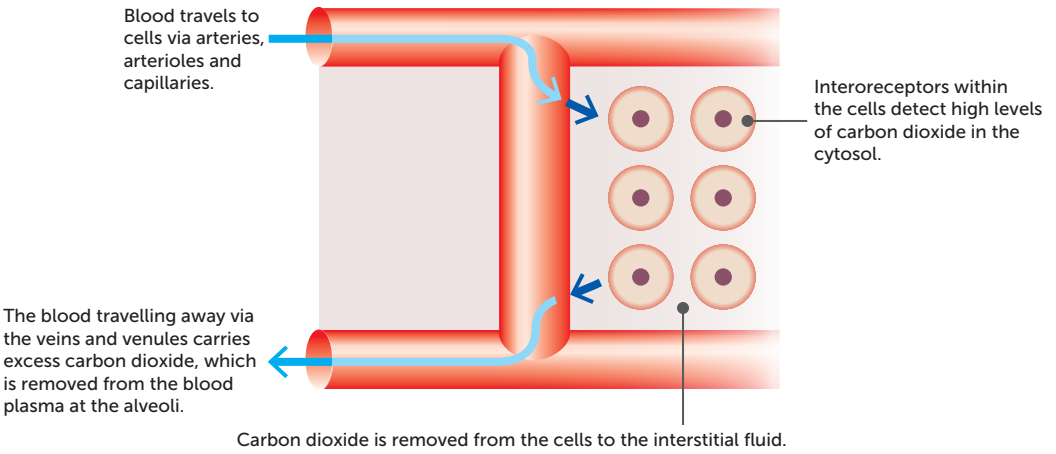

FIGURE 10.1 The exchange of carbon dioxide between blood plasma and interstitial fluid

The external receptors of organisms detect changes in their external environment, interpret these signals and coordinate a response for survival or development. External receptors can work as individual receptors or together as a group. They can be distributed evenly over the body (e.g. pain receptors), located in specialised areas (e.g. taste buds) or concentrated in organs (e.g. the eye).

TABLE 10.1 Some examples of receptors

TYPE OF RECEPTOR	STIMULI	LOCATION IN ANIMALS
Chemoreceptor	(Internal receptor) Detects oxygen and ion levels	Aorta, carotid arteries
Osmoreceptor	(Internal receptor) Detects changes in osmotic pressure in blood (changes in solute concentration in the blood)	Hypothalamus
Photoreceptor	(External receptor) Detects light	Eyes; light-sensitive cells on the body surface of some invertebrates
Thermoreceptor	(External receptor) Detects external temperature variations	Skin
	(Internal receptor) Detects internal temperature variations	Hypothalamus

Homeostasis has its limitations

CASE STUDY

In 1988, Mark Dorrity went on an 8 km run in extreme heat in New South Wales. During the run, his body overheated to 42.8°C and he neglected to drink water to stay hydrated. As a result, Mark's body could not regulate his temperature and water balance. His muscles generated more heat than could be lost from his body, and Mark suffered a rare condition known as rhabdomyolysis. His thigh muscles liquefied and released toxic proteins into his blood, causing kidney failure. Dehydration resulted in thickening of the blood to a point where it could not flow freely in some parts of the body. Every organ in his body was affected, he became delirious, brain damage occurred, his lungs barely functioned and his heart stopped. Within an hour, he collapsed into a 3-month coma, during which he was on dialysis and had one leg amputated due to gangrene.

Under normal conditions, Mark's body systems would have worked together to enable him to function comfortably, but in the extreme environmental conditions

his body's ability to maintain heat balance became impaired. His judgement overrode the warning signs, and his coordinating systems were unable to regulate his physiological responses to the heat. The conditions in his internal environment became intolerable. He is fortunate to have survived the experience.

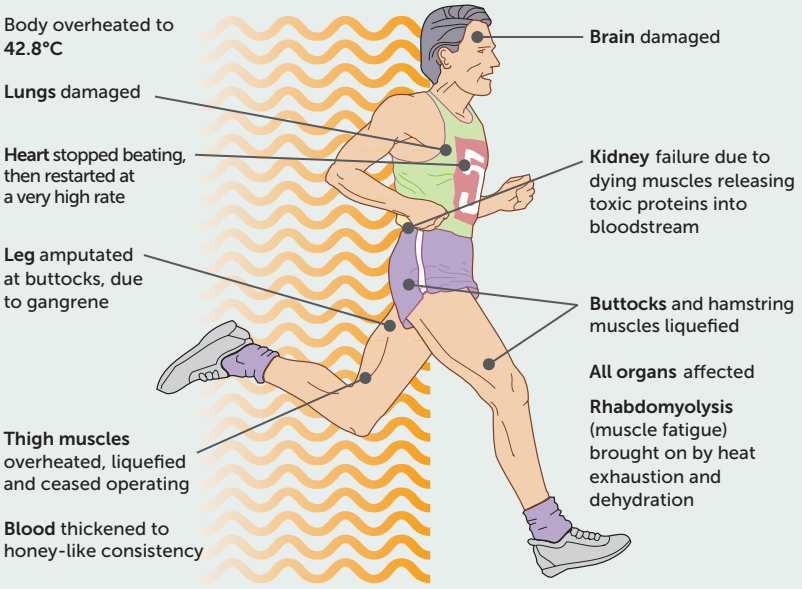

FIGURE 10.2 Extreme environmental conditions affect homeostasis with life-threatening consequences.

Coordinating a response

In complex animals, there are two systems (the **nervous system** and the **endocrine system**) that are responsible for monitoring changes, transmitting messages and coordinating responses. The nervous system is fast acting and the endocrine system is relatively slow acting.

The nervous system

The nervous system comprises the central nervous system (CNS) and the peripheral nervous system (PNS). The brain and the spinal cord form the CNS. These two structures receive sensory information from receptors, interpret and process the sensory information, and if a response is needed they coordinate the response. Sensory and **motor neurons** make up the PNS, which is responsible for transmitting information to and from the CNS.

Nerve impulses travel along defined pathways. They follow the **sensory neurons** from the source of stimulation, via the PNS to the CNS. **Interconnecting neurons** located in the CNS relay the electrical impulses from the sensory neurons to the appropriate motor neurons. From the CNS, motor neurons relay signals via the PNS to the **effectors** along different pathways. Effectors are the muscles or glands that respond to the stimuli.

Neurons are the basic units of the nervous system. Their structure is directly related to their function. They have extensions called fibres, along which nerve impulses travel. A bundle of nerve fibres comprises a nerve, and each nerve is wrapped in a tube of connective tissue.

Nerve fibres contain a tubular extension of the cell body, called an **axon**. The axon is enclosed in fatty material, functioning as **insulation**, called the **myelin sheath**. The myelin sheath is essential for proper nervous system function as it assists in the transmission of electrical impulses, speeding up transmission along the length of the nerve and preventing the message from crossing over to adjacent neurons.

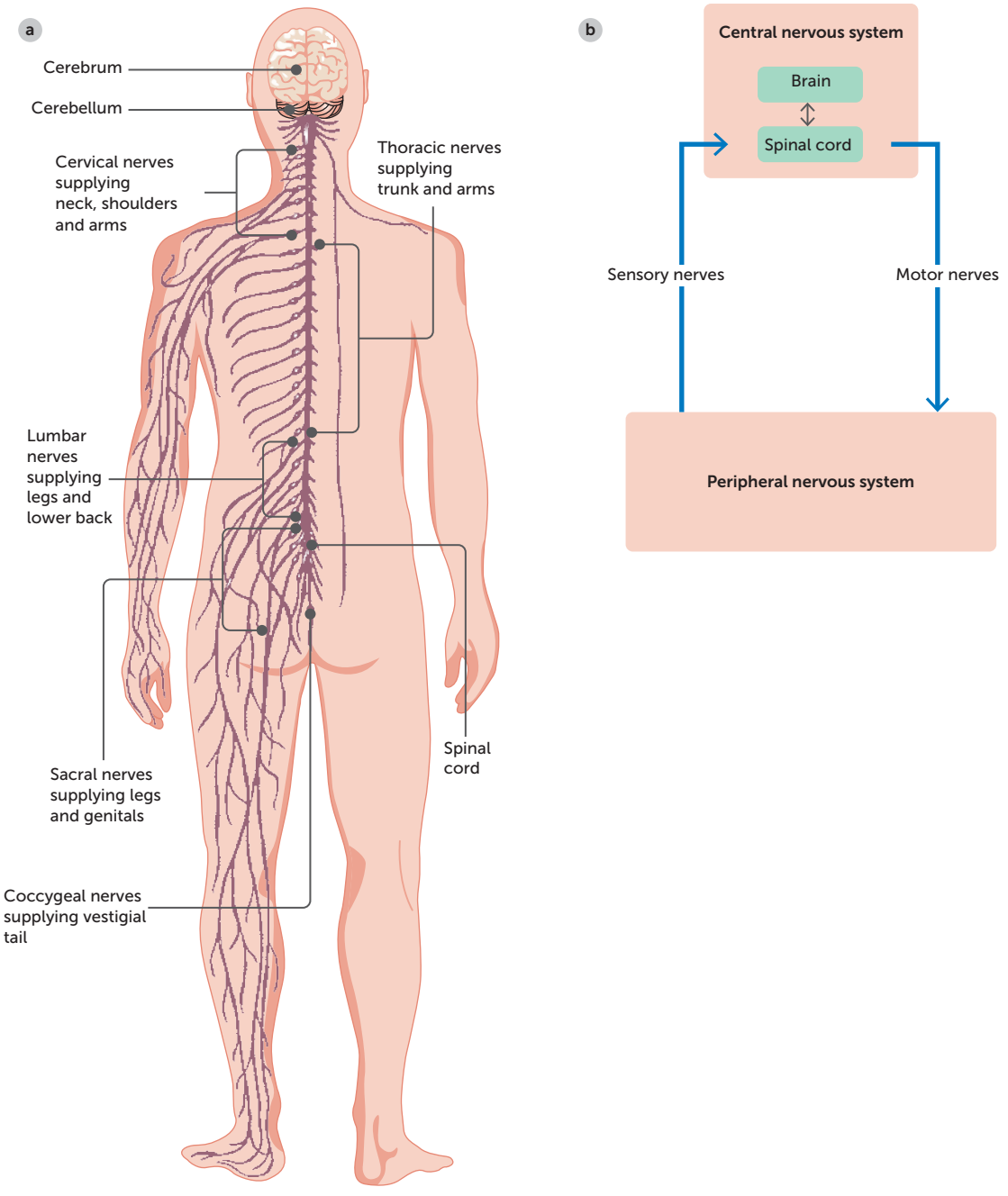

FIGURE 10.3 a Overview of the nervous system; **b** main divisions of the nervous system

Sensory neurons (in the PNS) transmit messages from the receptor organs to special regions of the brain (in the CNS) via the spinal cord. When one of the regions of the brain receives a message about a detected change, it coordinates any response necessary to counteract the change and sends messages to the effector organs (via the motor neurons in the spinal cord and then the motor neurons in the PNS).

Afferent and efferent are directional words. Messages sent in an afferent direction travel from the receptor cells to the CNS along (afferent) sensory neurons. Messages sent in an efferent direction travel from the CNS to the effector along (efferent) motor neurons.

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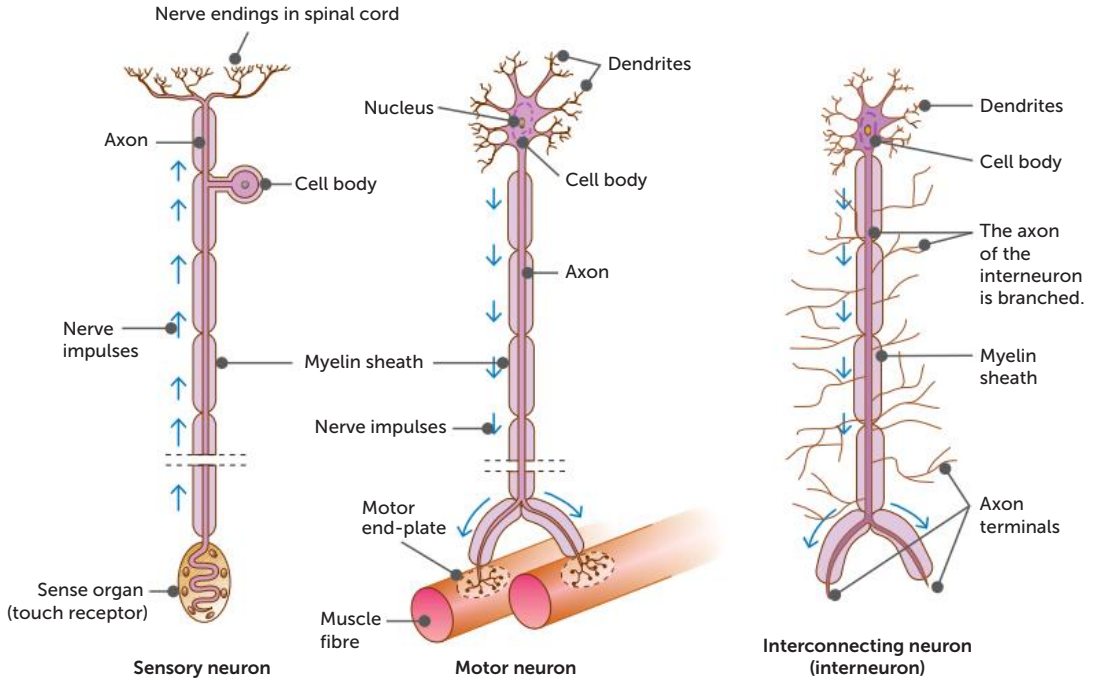

FIGURE 10.4 The generalised structure of sensory, motor and interconnecting neurons. All classes of neurons can have a variety of shapes.

The endocrine system

The hypothalamus and thermoregulation
View this tutorial and animation to reinforce your knowledge about the hypothalamus.

Not all changes in an organism's internal and external environment require an immediate response. Some responses take time and are under the control of hormones produced by the endocrine system.

Hormones are chemical substances, such as proteins, steroids, fatty acids and amino acids. In vertebrates they are secreted by ductless glands directly into the bloodstream. They target and activate particular cells and organs, causing a response (Table 10.2). Only the cells in the body that have receptors for a particular hormone will respond to that hormone.

TABLE 10.2 Examples of vertebrate endocrine glands, the hormones they secrete, and their function

ENDOCRINE GLAND	HORMONE SECRETED	TARGET TISSUE/ORGAN	FUNCTION RELATED TO HOMEOSTASIS
Posterior pituitary	Antidiuretic hormone	Kidney	Stimulates reabsorption of water
Thyroid	Thyroxine	Nearly all tissues	Increases metabolic rate, and as a result increases oxygen consumption and production of heat

A target tissue may be a long way from the gland that secretes the hormone (Figure 10.5). For example, antidiuretic hormone (ADH) is secreted by the **pituitary gland** in the brain but exerts its effects on the kidneys. It stimulates the reabsorption of water, helping maintain an appropriate water balance in the body.

The coordination of activities associated with the endocrine system is largely carried out by the pituitary gland. It is known as the master gland because it produces many hormones that affect hormone production by other endocrine glands. However, just above the pituitary gland is the hypothalamus, which controls the functioning of the pituitary gland in regard to water balance. The hypothalamus detects and coordinates many homeostatic factors.

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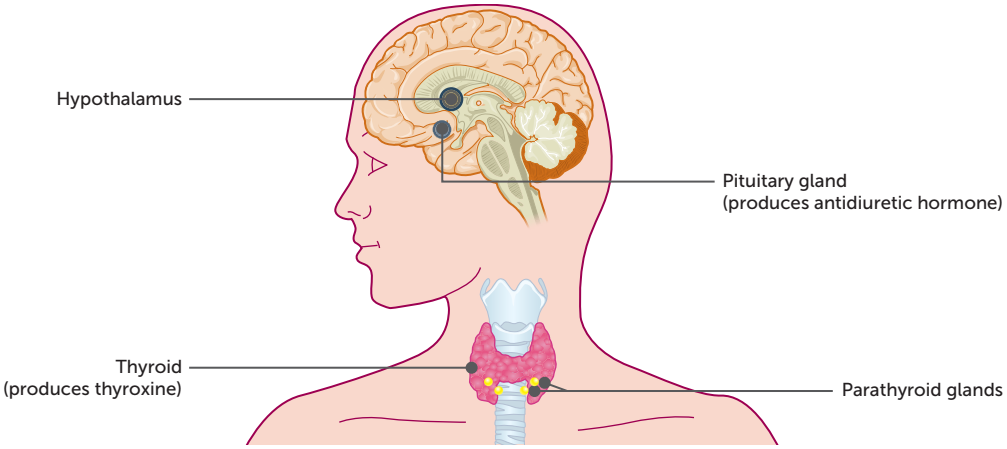

Homeostasis: body temperature
Watch this animation to learn about the function of the hypothalamus in temperature regulation.

FIGURE 10.5 Location of some of the endocrine organs in the human body

Hormones and hormone-like substances occur in other organisms and they are essential to the regulation of a variety of activities. Female ring doves coo during courtship to stimulate the release of the hormones that result in egg development. In plants, a light-sensitive hormone called auxin is responsible for plant growth towards light (**phototropism**) to maximise their photosynthetic ability.

Key concept

Homeostasis involves a stimulus–response method for maintaining a constant internal environment. The nervous system and the endocrine system are involved in detecting stimuli and transmitting a response. Sensory neurons in the PNS carry a message to the CNS, and motor neurons in the PNS transmit the message to effectors (i.e. muscles and glands). In the endocrine system, the hypothalamus detects changes and coordinates the response of hormones, many of which are produced by the pituitary gland.

Question set 10.1

REMEMBERING

1. Describe the difference between a stimulus and a response.
2. Draw and complete a table that summarises the different kinds of neurons. Use the headings: Type of neuron, Simple labelled diagram, Function.
3. Identify the systems of the body that are largely responsible for monitoring and coordinating response mechanisms.

UNDERSTANDING

4. Define homeostasis and outline its purpose.
5. Explain why it is important for an organism to be able to detect changes in its external environment. Give an example.
6. Clarify the difference between an internal receptor and an external receptor. Name an example of each.

APPLYING

7. Explain why it is important for the hypothalamus to detect changes in temperature and osmotic pressure (water balance in the blood) in mammals.

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10.2 NEGATIVE FEEDBACK: THE MECHANISM FOR MAINTAINING HOMEOSTASIS

Homeostasis and feedback mechanisms
Watch these videos to reinforce your understanding of homeostasis and compare negative and positive feedback.

The external environment can vary greatly in terms of temperature, water availability, nutrients, oxygen and carbon dioxide. The fluctuations in these factors, and others, can cause internal factors to deviate away from their optimal values. Organisms have narrow ranges within which they need to keep their internal temperature and fluid concentrations. Complex organisms have strategies and homeostatic mechanisms to keep internal conditions relatively stable. This enables the necessary biochemical processes to be maintained.

Signals from receptors are sent to a **coordinating centre (modulator)**. The coordinating centre interprets the signals and coordinates a specific response. A response that leads to homeostasis is one that counteracts (gives negative feedback about) the stimulus. Less common are responses that reinforce (give **positive feedback** about) the deviation from the optimal state. These processes are referred to as **feedback mechanisms**. A feedback mechanism is triggered when a stimulus is detected by a receptor. The information about the stimulus is then processed, and a message is conveyed to an effector, which carries out a physiological response to the stimulus.

In analysing how cells and organisms respond to signals, it is useful to apply a stimulus–response model. This model represents the feedback mechanisms. In animals, the main body organs that respond to signals are glands or muscles. Since the receptor organs are different from the effector organs, communication between cells to coordinate a response is essential.

Feedback mechanisms are physiological processes that respond to small disturbances to keep internal conditions and concentrations of substances within narrow limits for optimal function.

Negative feedback

A homeostatic process that responds by changing the direction of a stimulus is a negative feedback loop. The initial stimulus is deviation of a factor away from normal. A negative feedback loop will always reduce the stimulus. Many animals are regulators and employ negative feedback loops to maintain their internal environment. For example, a platypus will regulate its body temperature in the water to counteract changes in the external environment.

For the platypus to maintain its body temperature, certain mechanisms need to be used to return the body temperature to normal. Negative feedback is extremely important in homeostasis, because the response always works to restore the internal environment to a constant set of conditions. Negative feedback is a mechanism that stabilises internal conditions.

Negative feedback can be modelled using a flow diagram. To understand the flow diagram, the following terminology is helpful.

TABLE 10.3 Parts of a negative feedback model and the roles they play

PART	WHAT IT IS AND WHAT IT DOES
Stimulus	A change in one of the internal or external environmental factors that is detected by a receptor. The change involves a deviation away from the normal or optimal value.
Receptor	The cell or tissue that detects the stimulus (change in the environment). The receptor may be internal or external.
Coordinating centre (modulator, usually the hypothalamus)	The structure that receives messages from receptors (via sensory neurons), coordinates a response, then sends an instruction to an effector via motor neurons.
Effector	A muscle or gland that receives the message from the control centre and carries out the response.
Response	The action of the effector that counteracts the stimulus (i.e. the change made by the effector).
Negative feedback	A message that counteracts the stimulus; it returns the value back to its normal or optimal value.

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Homeostasis and feedback

Homeostasis
Reinforce your knowledge about homeostasis by watching this video.

Figure 10.6 provides a generalised overview of homeostasis and feedback mechanisms. The model is represented as a cycle, but in reality, the mechanism is only cyclic if a stimulus continuously occurs after negative feedback.

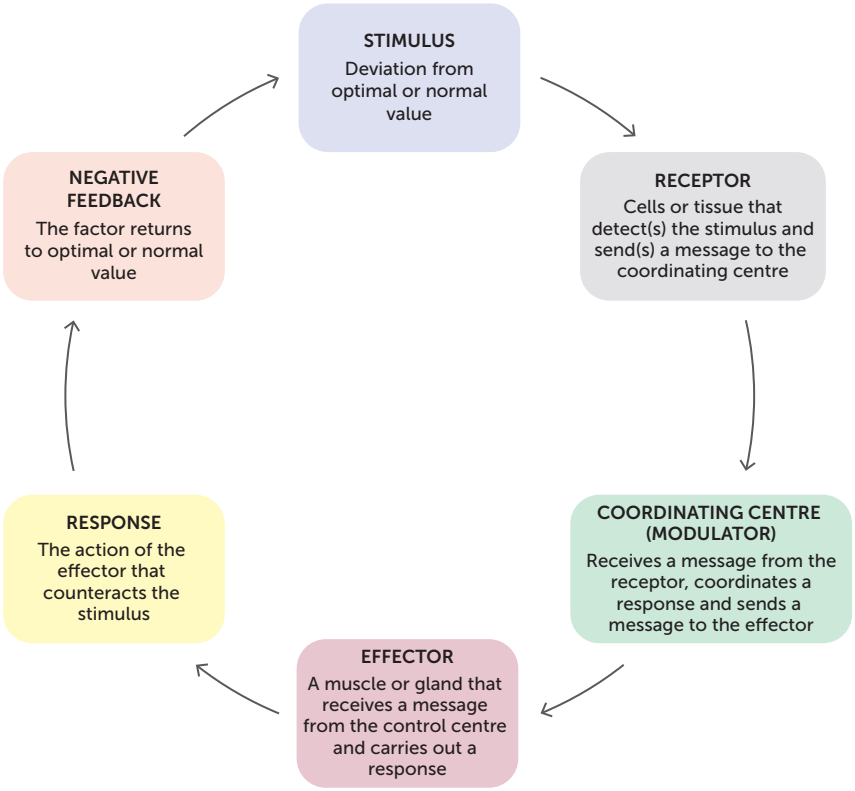

FIGURE 10.6 A generalised negative feedback loop

This negative feedback model (loop) is an example of a specific factor that can increase or decrease in its deviation away from normal, but is regulated using negative feedback mechanisms.

Positive feedback

If a response reinforces the original stimulus, the mechanism is referred to as positive feedback. This process increases the output of the system, further enhancing the deviation from the optimal or normal value. Positive feedback is rare but necessary during some developmental processes. For example, the development of frogs and toads is controlled by the hormone thyroxine. Just before the tadpole changes (metamorphoses) into an adult frog, negative feedback is changed into positive feedback. The concentration of thyroxine rises and it triggers metamorphosis. Other examples include blood clotting (platelets releasing clotting factors, which cause more platelets to aggregate at the site of injury) and lactation (the feeding child stimulating breast-milk production, which causes further production that continues until the baby stops feeding).

In terms of homeostasis, positive feedback can be harmful. When human body temperature rises during a fever, a new and higher **set point** for temperature can be established, and the person may suffer from heatstroke. If the resetting of the set point continues upwards, cell function is impaired.

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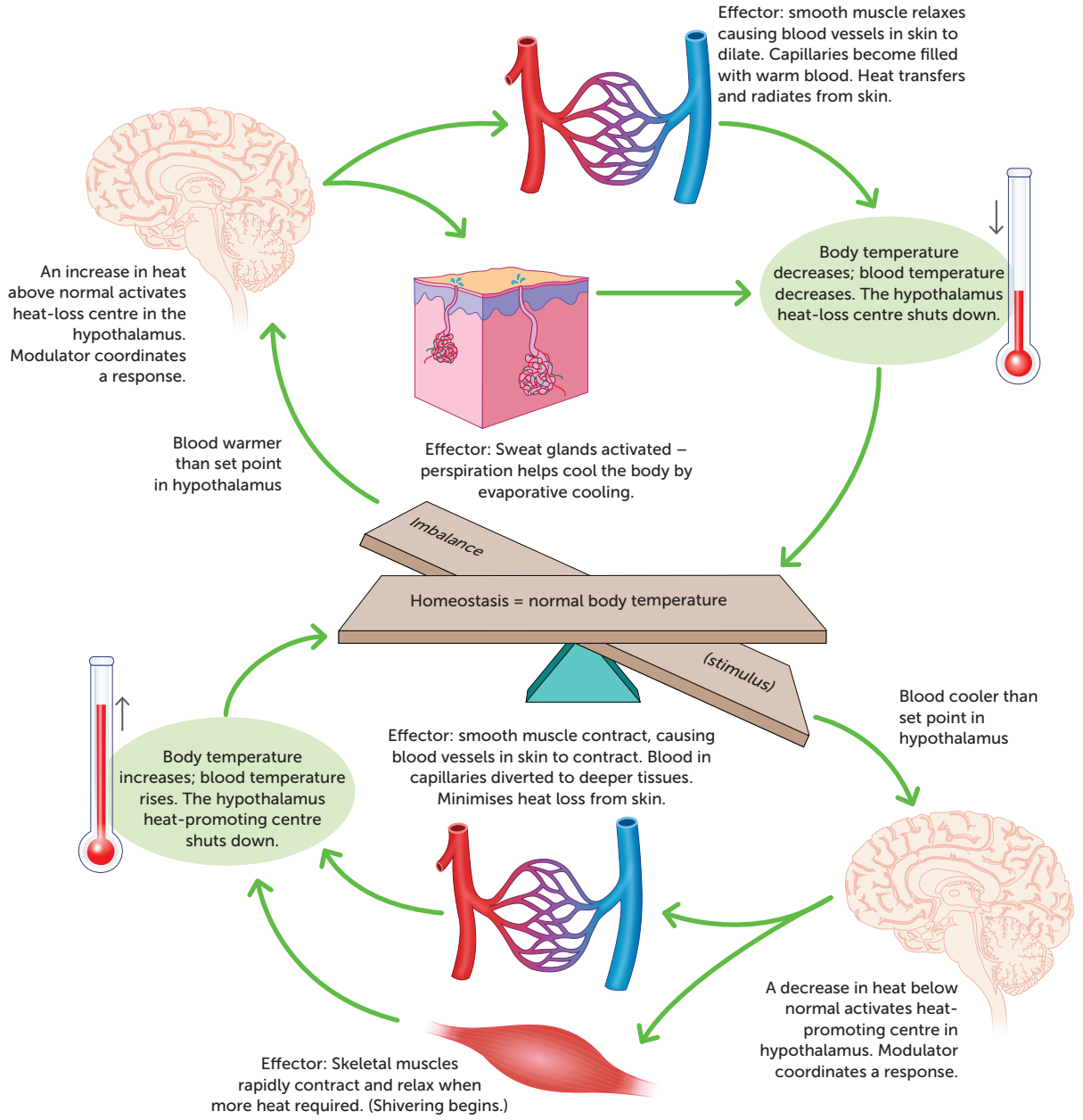

FIGURE 10.7 Negative feedback loop for an increase or decrease in body temperature in mammals

Skin structure and function
Learn more about skin structure and function

Key concept

Feedback mechanisms are triggered by a receptor detecting a stimulus. The information is processed by a modulator, and a message is transmitted to an effector to carry out the response. Feedback can be positive (reinforce the stimulus) or negative (reduce the stimulus). Negative feedback is the main mechanism for maintaining homeostasis.

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Question set 10.2

REMEMBERING

1 Outline the stimulus–response model.
2 Define:
   a stimulus
   b receptor
   c effector
   d response.

UNDERSTANDING

3 Using an example, explain negative feedback.
4 Clarify how positive and negative feedback differ. Suggest why positive feedback is generally not associated with homeostasis.

APPLYING

5 Draw a stimulus–response model that demonstrates how temperature levels are maintained in the body.

10.3 TOLERANCE LIMITS

Each organism has set ranges within which they can tolerate the temperature, water balance and different levels of organic and inorganic materials. These are known as **tolerance ranges** (Figure 10.8). Organisms can survive change in a factor if it remains within their tolerance range. If a factor goes outside of the tolerance range, it may be fatal for the organism.

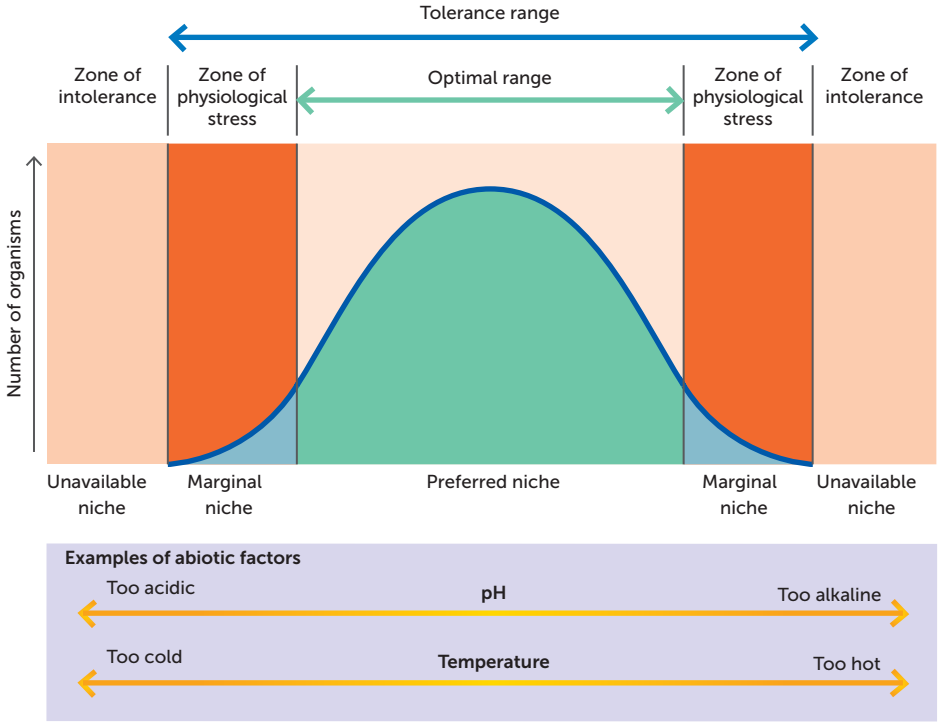


FIGURE 10.8 The optimal range for an abiotic factor is the range within which an organism functions best. Organisms can also function in the zone of physiological stress, but not in the zone of intolerance.

For example, dissolved oxygen in the ocean allows many aquatic animals to survive. Fish, crabs, oysters and other aquatic animals need sufficient levels of dissolved oxygen in the water. The amount of dissolved oxygen in an **estuary**'s water is the major factor that determines the type and abundance of organisms that can live there. When levels of oxygen are within the tolerance range but outside the **optimal range** this is known as the **zone of physiological stress**. Marine life such as crabs and fish can survive in zones of physiological stress, but they will be lethargic because their respiration rate will be lower. Oxygen levels below the tolerance range will not be high enough for a lot of marine life to survive; this is the **zone of intolerance**. When air and water temperatures increase or there are high

levels of decomposition of algal blooms, this can lead to oxygen depletion. Many animals and plants cannot survive when the dissolved oxygen falls to such low levels.

Homeostatic regulation seeks to maintain levels within the optimal ranges. The optimal range is the set of values for a factor that enables an organism to function best, in its healthiest state, with optimal growth and reproduction. The optimal range falls within the tolerance range. If oxygen levels in an estuary are within the optimal range, fish, crabs and other marine life can potentially respire at their maximum rates possible, leading to high metabolic, growth and reproductive rates.

Key concept

An organism's tolerance range is the range of external environmental conditions within which it can survive and maintain homeostasis. The tolerance range includes the optimal (preferred) range and the zone of physiological stress (less preferred). Outside this range (in the zone of intolerance) many animals and plants cannot survive.

Effects of moving outside tolerance limits

Any deviation outside the tolerance limits can affect the functioning of cells and the health of the organism. There are many potential effects for cells and living organisms when major factors deviate from the tolerance range.

If homeostasis fails and the level of pH, for example, becomes too high, an organism can fall into a state of **physiological stress**, affecting its function. Internal factors that have a tolerance limit include temperature, **nitrogenous waste**, water, salts, and gases. These factors need to be regulated to stay within their tolerance ranges to enable cells to live, reproduce and function.

Temperature

Homeostasis: blood sugar and temperature
Recall the effect of temperature on enzyme activity and therefore on metabolic activity rate.

A small increase in temperature can cause an increase in enzyme (protein) activity. This increases the metabolic rate, because enzymes are biological catalysts. However, a significant increase (to 40–50°C in mammals) can cause enzymes to denature, leading to critically slow cell metabolism. Cells can die. When human body temperature reaches 37.7–39.2°C, cognitive skills start to decline. The person experiences heat stress, heat exhaustion, and finally heat stroke (if temperature increases to 39.2–39.6°C). At high temperatures, cell membranes become too fluid, allowing some unwanted substances into cells, or wanted substances out of cells. In plants, photosynthesis can slow down significantly when enzymes denature. This affects plant growth and productivity.

A decrease in temperature below the optimal range can lead to a decrease in enzyme activity, which results in a decrease in metabolic rate. The action of some other proteins may decrease as well. For example, uptake of oxygen by the haemoglobin protein can be less effective, leaving animals feeling sluggish. At low temperatures, cell membranes can become rigid (rather than fluid), slowing the transport of substances across them.

When there is a decrease in temperature below the tolerance range, mammals can suffer **hypothermia** and sometimes even lose limbs. Some animals may survive but cannot reproduce until temperatures return to within their tolerance range.

Nitrogenous waste

Proteins and nucleic acids are required by organisms for survival. They both contain nitrogen. When organisms break down proteins and nucleic acids, a highly toxic nitrogenous waste is formed, known as **ammonia**. Most terrestrial animals convert this to **urea**, but urea still needs to be dissolved in water and excreted because it is moderately toxic. (The nitrogenous waste that is least toxic is **uric acid**, which requires very little water for its excretion but is made at an energy cost.) As nitrogenous wastes increase in concentration, they become more toxic.

An increase in ammonia in the blood can lead to an increase in pH. Enzymes can only function within a certain pH tolerance range, and they perform at their peak within an optimal pH range. When

the cellular pH is outside the optimal range, enzyme activity can decrease. When the cellular pH is outside the tolerance range, the enzymes can denature, resulting in critically slow metabolism.

High levels of nitrogenous waste can also affect the water balance. Cells may lose water to dilute the waste in an attempt to maintain pH homeostasis.

Water

Water is the universal **solvent**. It dissolves salts and minerals by breaking salts into ions. For example, sodium chloride (NaCl) can be dissociated (dissolved and separated) into Na^+ and Cl^- ions. The ions are then ready to partake in metabolic processes. Metabolic reactions occur in a solution composed mostly of water. An increase in water content (and a decrease in ion concentration) leads to a decrease in the collision rates of the reactants involved in the biochemical pathways, slowing metabolism. Too much water results in an inability to regulate the concentration of solutes such as salts.

The movement of water across a semipermeable membrane from an area of high concentration of water (low concentration of solute) to an area of low concentration of water (high concentration of solute) is called osmosis. An increase in water content to above the tolerance range will lead to a **hypotonic** (lower than normal solute concentration) solution surrounding the cells in the blood and in the tissues. Water will then move into the cells down a concentration gradient in order to reach equilibrium. If too much water enters the cells, animal cells can swell and burst (cell lysis), and plant cells can swell. If cells swell, the resulting solute concentration can be too low, leading to a decrease in collisions of reacting particles, slowing the rates of reactions. Thus, if the concentration inside cells becomes too dilute, there are insufficient interactions between enzymes and **substrates**.

Too little water inside cells also results in an inability to regulate the concentration of solutes such as salts. A **hypertonic** (higher than normal solute concentration) solution surrounding cells in the blood and in the tissues may lead to water moving out of the cells by osmosis. This leads to dehydration. Cells can shrink and plant cells can undergo **plasmolysis**. Plasmolysis is the term used when the cell membrane of a plant cell has pulled away from the cell wall due to water moving out of the cell. In an environment that has a water content below the tolerance range, ions are unable to move to their reaction sites at a fast enough rate, slowing the metabolic rate. The cells are then unable to regulate the concentrations of solutes (leading to an increase in osmotic pressure). Toxic waste cannot be excreted effectively, leading to an increase in pH, which affects enzyme activity. In animals, blood plasma is 90% water. It transports nutrients to cells and waste products away from cells. A decrease in water content can slow the rate of transportation. When cells are surrounded by fluid of the same water concentration, the surrounding solution is described as **isotonic** and there is no net movement of water.

Salts

Salts dissociate (dissolve and break apart) into ions. Ions such as sodium (Na^+) and calcium (Ca^+) are required to be in concentrations within a fairly narrow range for normal activity of muscles, neurons (nerve cells) and other body cells. If the salt concentration increases on the outside of cells, water may be transported out of the cells by osmosis. This leads to cell shrinkage and dehydration. Salts are needed at optimal levels in order to regulate water balance.

Calcium, for example, triggers muscles to contract. Low calcium levels in the blood can lead to cramping in the legs and back. Low blood sodium levels affect water balance, blood pressure and the nervous system. If the Na^+ concentration drops too low outside cells, water moves into the cells, the cells swell with too much water, and this can lead to weakness, fatigue and confusion.

Gases

Living cells need a continuous supply of oxygen in order to carry out respiration for energy production. Carbon dioxide is a waste product of respiration. It readily dissolves in water, forming carbonic acid (H_2CO_3), and then further dissociates to form hydrogen ions (H^+) and bicarbonate ions (HCO_3^-). High levels of carbon dioxide can lead to a high concentration of H^+ ions in solution, which lowers the pH of the blood, making it more acidic. pH is a measure of how acidic or alkaline (basic) an aqueous

solution is. It is also a measure of the hydrogen ion concentration. Solutions with a pH of 7 are neutral. If the pH is above 7 the solution is alkaline, and if the pH is below 7 the solution is acidic. In the case of mammals, the blood pH lowers when the carbon dioxide level is high. The excess carbon dioxide needs to be removed as quickly as possible, because this lowering of pH affects homeostasis and can reduce enzyme activity rate or even denature enzymes. If the carbon dioxide level gets too low, it leads to a lower ventilation (breathing) rate in animals and a lower rate of photosynthesis in plants.

It can also be toxic when oxygen increases above the tolerance range in animals. The gas can diffuse straight into cells. A high level of oxygen can cause cell damage, nausea, dizziness and breathing problems. If, on the other hand, the oxygen gets too low, this leads to a reduction in the respiration rate and thus the rate of ATP (energy) production.

TABLE 10.4 Summary of potential effects on organisms when internal factors deviate away from their tolerance ranges

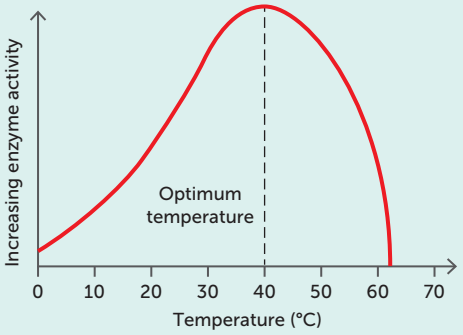
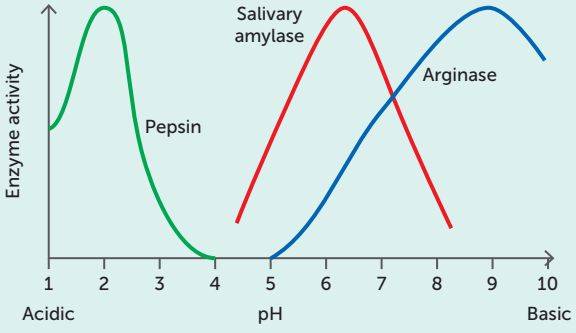
INTERNAL FACTOR	AN INCREASE	A DECREASE	VISUAL AID
Temperature	**1** Enzymes (proteins) denature, leading to critically slow cell metabolism. **2** Cells can die. **3** Membranes such as cell membranes become too fluid, allowing some unwanted substances into or wanted substances out of cells. **4** Rate of photosynthesis slows.	**1** Decrease in the activity rate of enzymes, which results in a decrease in metabolic rate. **2** The activity of some other proteins decreases. **3** Membranes such as cell membranes become rigid (instead of fluid), slowing cell membrane transport of substances. **4** Mammals can suffer hypothermia, may lose limbs and cannot reproduce.	 As temperature increases so does the rate of reaction. However, at high temperatures, some of the bonds that hold the enzyme together break, changing the shape of the active site so the enzyme becomes denatured.
Nitrogenous waste	**1** As nitrogenous wastes build up, they increase in concentration and become more toxic. **2** An increase in ammonia (a base) in the blood can lead to an increase in the pH of body fluids. **3** Enzyme activity can decrease; enzymes will denature if the pH gets too high. **4** High levels of nitrogenous waste can affect water balance. Cells may lose water to dilute the waste, affecting water homeostasis.	Not applicable	 Different enzymes work best at different pHs. If the pH changes too far from this optimum, the bonds holding the enzyme together weaken, changing the shape of the active site so the enzyme becomes denatured.

FIGURE 10.9 Summary of potential effects of temperatures outside the temperature tolerance range

FIGURE 10.10 Summary of potential effects of pH outside the pH tolerance range

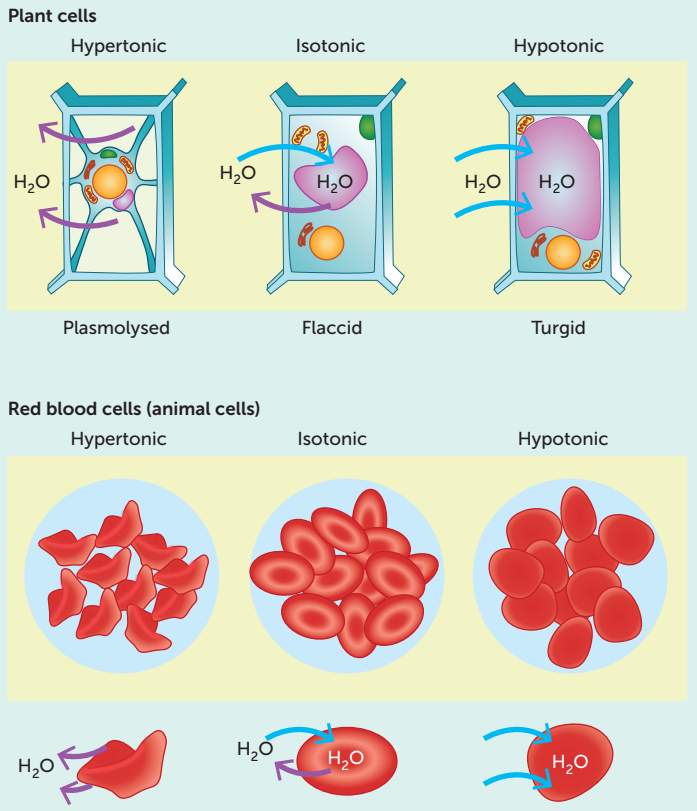
INTERNAL FACTOR	AN INCREASE	A DECREASE	VISUAL AID
Water	**1** Too much water results in the inability to regulate salt and other solute concentrations. **2** An increase in water concentration leads to a decrease in the collision rates of reactants involved in biochemical pathways, slowing metabolism. **3** An increase in water above the tolerance range leads to a hypotonic solution. Animal cells can swell and burst (cell lysis); plant cells can swell. **4** Solute concentrations can be too low, leading to a decrease in collisions of reactant particles, slowing the rate of reactions, as in the case of excess water concentration.	**1** Too little water results in the inability to regulate salt and other solute concentrations. **2** A hypertonic solution can surround the cells, leading to movement of water out of the cells by osmosis. **3** Dehydration – cells can shrink, and plant cells can undergo plasmolysis. **4** Ions are unable to move to their reaction sites fast enough; metabolic rates slow down. **5** Toxic waste cannot be excreted effectively, leading to increased pH, which affects enzyme activity. **6** Blood plasma is 90% water in animals. A decrease in water content can slow the rate of transportation of nutrients and wastes.	

FIGURE 10.11 The effects of hypertonic, isotonic and hypotonic solutions on plant cells and red blood cells (animal cells)

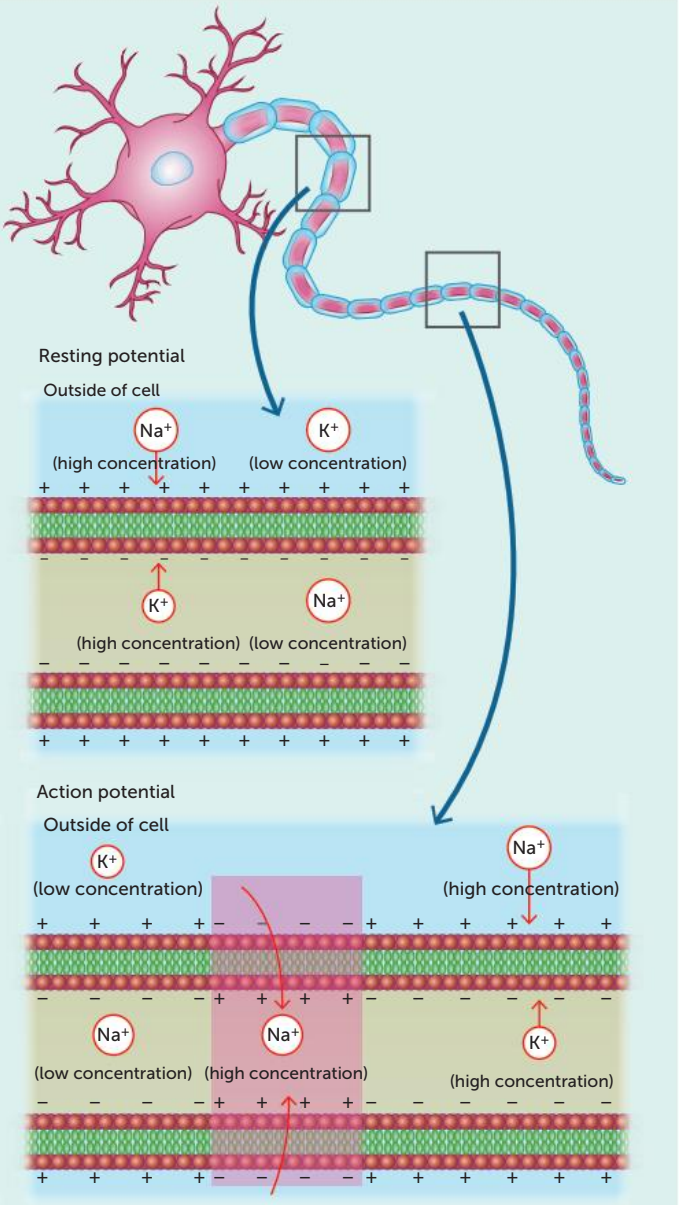
INTERNAL FACTOR	AN INCREASE	A DECREASE	VISUAL AID
Salts	**1** Salt ions such as Na^+ and Ca^+ are required within fairly narrow limits for normal activity of muscles, neurons and other body cells. **2** As salt concentration increases, water may be transported out of the cells by osmosis. This leads to cell shrinkage and dehydration. **3** High levels of Na^+ disenables regulation of salt concentrations and therefore water balance. **4** Higher than normal levels of potassium (K^+) can impair the function of skeletal muscles, the nervous system and the heart. High levels can cause excitation of muscle and nerve cells and cause muscle cells to lose the ability to relax.	**1** Low Ca^+ levels can lead to muscle cramping in the legs and back. **2** Low blood Na^+ levels affect water balance, blood pressure and the nervous system. **3** If the Na^+ concentration outside cells is lower than inside, water moves into cells. Cells can swell with too much water. Swollen red blood cells can lose their oxygen-carrying efficiency. This can lead to weakness, fatigue and confusion.	

FIGURE 10.12 Salts are needed at optimal levels for the normal activity of neurons.

What if you drink saltwater?
How does drinking salt water affect our cells?

INTERNAL FACTOR	AN INCREASE	A DECREASE	VISUAL AID
Gases	1 High levels of CO_2 can lead to a high concentration of H^+ ions in solution, which lowers pH. 2 Lowering of pH affects homeostasis and can reduce enzyme activity rate or denature enzymes. 3 When the O_2 level increases above the tolerance range in animals, this can be toxic. It can cause cell damage, nausea, dizziness and breathing problems.	1 A reduction in O_2 leads to a reduction in the respiration rate and the rate of ATP (energy) production. 2 Low CO_2 leads to lowered ventilation (breathing) rate in animals and a lower photosynthesis rate in plants.	 Shutterstock.com/Patryk Kosmider **FIGURE 10.13** Low blood oxygen levels can cause French bulldogs to develop blue gums (due to their short nose and narrow nostrils).

Adaptations maintain an organism's internal environment within tolerance limits

Adaptations are features and strategies that help organisms survive in a particular environment, including by maintaining their internal environment within tolerance limits. The three main types of adaptations are physiological processes, structural features and behavioural adaptations. Physiological adaptations are related to how an organism, system, organ, tissue or cell functions. Structural adaptations are related to an organism's shape, specialised features and size. Behavioural adaptations relate to how the organism acts.

Metabolism is the sum of the chemical reactions that occur within an organism to maintain life. The majority of these reactions require the catalytic help of enzymes. The various enzymes function best at particular pH levels, solute concentrations and temperatures. Metabolic activity is not only responsible for the breakdown or synthesis of molecules; it also creates internal body heat. An increase in metabolic activity increases internal temperature as a result of the energy released in the reactions, and a decrease in metabolic rate decreases the internal temperature. However, there are other factors that alter temperature and pH levels. For example, concentration of carbon dioxide in the internal environment can alter pH levels. If carbon dioxide concentrations increase as a result of exercise, pH levels decrease. Decreasing the pH causes lower enzyme functionality. In turn, metabolic activity is reduced, resulting in less heat energy being produced. The body must maintain pH levels to ensure the supply of nutrients to cells is met and the internal temperature remains constant.

Physiological processes

Physiological processes are the functional processes performed by organisms. Cells, tissues and organs perform functions to help maintain homeostasis. Physiological processes maintain a balanced internal environment through monitoring and adjusting as conditions change. The physiological processes involve the three main parts of a negative feedback system – receptor, control centre and effector.

As muscles contract and release more rapidly, the demand for oxygen and glucose increases. These two reactants are substrates for cellular respiration and supply cells with energy. Carbon dioxide is a by-product of this **catabolic reaction**, along with heat. As activity increases, the carbon dioxide levels and internal temperature rise. Physiological mechanisms are in place to minimise these changes. One way to reduce carbon dioxide concentration is by increasing the breathing rate. This mechanism passes more blood through the lungs, releasing the carbon dioxide into the external environment. The blood is also oxygenated faster, which maintains cellular respiration throughout the activity.

The changed internal temperature is detected by thermoreceptors in the hypothalamus, which signals to the sweat glands to become active, removing excess body heat.

Structure and behaviour

Structural features and behaviour also play a part in maintaining an organism's internal environment within tolerance limits. Structural features are physical features that usually have a function. They include cell structure and the size and shape of an organism. Structural adaptations are special biological structures that assist in homeostasis; for example, the thick insulating fur that keeps a bear warm; the thin, flat leaves of a tree that maximise sunlight capture by chloroplasts; and the vast capillary network over the alveoli that creates a large surface area for carbon dioxide and oxygen exchange to work efficiently.

The behaviour of an organism can also help maintain its internal environment. Such behaviour is usually an action performed in response to a stimulus. Burrowing in mud helps desert-dwelling frogs avoid desiccating (drying out). The moist, delicate skin of a frog can dry out easily, preventing it from achieving adequate gas exchange and leading to death. Much of WA is classified as desert, and to live in those areas the water content of frogs must stay within their tolerance range. The desert spadefoot's behaviour of burrowing underground during the dry months prevents exposure to the sun while it waits safely for rainfall. In humans, the removal of clothing or moving into the shade are behaviours seen when an individual notices an increased body temperature. In addition, exercise pace will slow, slowing the breathing rate and the removal of carbon dioxide. These actions change the metabolic rate and the heat energy produced.

Getty Images/Auscape International Pty Ltd

FIGURE 10.14 The desert spadefoot has moist, delicate skin but lives in the desert.

Agefotostock/ Mint Frans Lanting

FIGURE 10.15 A desert spadefoot emerges from its burrow underground.

Key concept

Homeostatic adaptations that work to maintain tolerance limits can be physiological, structural or behavioural.

Question set 10.3

REMEMBERING

1 Describe the effect of each of the following on cells when they rise above the optimal range:
   a temperature
   b concentration of nitrogenous wastes
   c oxygen
   d water
   e salt.
2 What are the by-products of cellular respiration?

UNDERSTANDING

3 Differentiate between tolerance range and optimal range.
4 Explain the difference between cellular respiration and metabolic activity.

APPLYING

5 Explain why metabolic rate can drop significantly when temperatures become significantly higher than the limit of the tolerance range.

10.4 THERMOREGULATION

Thermoregulatory mechanisms also include structural features, behavioural responses and physiological adaptations to control heat exchange and metabolic activity. Animals and plants must control heat exchange and metabolic activity in order to survive. Life is found over a broad range of temperatures in the biosphere of Earth, which can vary from −75°C to above 50°C. However, most individual species can only survive within a relatively narrow range of temperature, and many cannot exist in habitats that have greatly varying temperatures. Their structural, behavioural and physiological adaptations help them to maintain their temperature within this narrow range. Within their tolerance range, each species has an optimal temperature range at which it functions best. Thermoregulation is critical for survival, because most biochemical and physiological processes are temperature sensitive. For every 10°C increase in temperature, most enzyme-mediated reaction rates decrease by 50–65%.

Some mechanisms that plants use to control heat exchange and metabolic activity are listed in Table 10.5.

TABLE 10.5 Features that help plants control heat exchange

COLD CLIMATES	HOT CLIMATES
 Alamy Stock Photo/Avalon/Photoshot License	 Alamy Stock Photo/Image Professionals GmbH
FIGURE 10.16 The Australian snow gum (*Eucalyptus pauciflora*)	**FIGURE 10.17** The pink spike hakea (*Hakea coriaceae*)
Its thick, leathery, waxy leaves reduce heat loss by providing insulation against the cold climate.	Its narrow, vertical leaves minimise the amount of direct sunlight hitting them and thus minimise heat absorption during hot desert days. (The leaves also increase the hakea's resistance to cold temperatures and frosts, which deserts can experience at night.)

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Different animals have different optimal internal temperatures. These are the temperatures at which their enzymes work efficiently. In mammals, if internal temperatures rise much above the set point, the enzymes denature, metabolic processes fail and the individual suffers from **hyperthermia**. Conversely, if body temperatures fall, enzyme function slows significantly and the individual suffers from hypothermia. Organisms use a variety of thermoregulatory processes to maintain homeostatic internal body temperatures.

Types of thermoregulation in animals: ectothermy and endothermy

Ectotherms and endotherms
Watch this video to find out about Australian endotherms and ectotherms.

Heat for thermoregulation can either come from metabolism or from the external environment. Animals that use metabolic processes to generate their own heat to maintain their internal temperature within the tolerance range are called **endotherms**. They have a range of adaptations that serve as mechanisms for controlling heat gain or loss. Animals such as birds and mammals can generally maintain a stable internal temperature independent of external temperature fluctuations. In a cold environment, an endotherm generates enough heat to keep its body within its tolerance range and at temperatures significantly higher than its surroundings. In a hot environment, such as in the Australian desert during the day, endothermic vertebrates use special mechanisms to keep cool. In addition to birds and mammals, there are some reptiles, fish and insects that are mainly endothermic.

In contrast, amphibians, some reptiles and fish, and most invertebrates cannot maintain a stable internal environment. An animal whose body temperature is determined by the external environment is called an **ectotherm**. Ectotherms rely on external sources for gaining heat. To gain heat, ectotherms may obtain heat from the sun or from objects in their surroundings. This means their body temperature fluctuates with the external environment. Ectotherms have adaptations that help in controlling heat gain or loss to regulate temperature, but these are structural or behavioural rather than physiological.

Endotherms and ectotherms use a variety of adaptations to regulate internal temperature. Some endothermic moths and beetles can raise their body temperature for short periods by vigorous flapping of their wings, generating heat by muscular activity. These and many other animals (such as mammals, birds and fast-swimming fish like yellowfin tuna) retain the heat generated by metabolic activity within their bodies. Further examples of endotherms are kookaburras, penguins, emus, koalas, wombats and humans. Ectothermic animals include desert lizards, tropical marine invertebrates (e.g. blood lobster, sea apple, cleaner shrimp), the desert pupfish, snakes, lizards, frogs, the majority of fish, and many invertebrates (e.g. spiders, starfish, snails).

Scientists avoid using the terms 'warm-blooded' and 'cold-blooded' because they are misleading. For example, if an ectothermic lizard is basking in the sun, its body temperature may be 'warmer' than an endothermic seal that is using faster metabolism to warm up.

Shutterstock.com/Zoe Ezzy

FIGURE 10.18 Crocodiles are ectothermic. Behaviours such as mouth-gaping and moving in and out of the water help them thermoregulate.

TABLE 10.6 Comparing the costs and benefits of endothermic and ectothermic thermoregulation

	ENDOTHERMS	ECTOTHERMS
Cost	To maintain a stable internal temperature, they may have a higher metabolic rate. They need to spend more energy to maintain a higher metabolic rate. This results in higher food requirements and more time spent finding food.	Body temperature is dependent on the external environment. These animals are limited to living in environments with less extreme temperatures. They cannot tolerate very high or very low external temperatures.
Benefit	Body temperature is independent of external temperature. This enables endotherms to live in more extreme environments. They can be active at night (when some ectotherms are not) or more often during the day and in cold weather. Being more active may reduce the chance of predation.	Their heat source is mainly the environment, so there are lower energy requirements for these animals. Therefore, they need to consume less food. They can spend less time hunting for food. They can tolerate larger fluctuations in their internal body temperature compared with endotherms.

Key concept

Animals can be described as endothermic or ectothermic. Endotherms are able to generate their own heat to maintain their internal temperature. Ectotherms cannot maintain their own internal temperature and rely on external sources for gaining and losing heat.

Question set 10.4

REMEMBERING

1 List three endotherms and three ectotherms.
2 Recall three main groups of thermoregulatory mechanisms.

UNDERSTANDING

3 Explain the differences between endotherms and ectotherms, and name examples of each.

CREATING

4 Create a debate to argue whether endotherms or ectotherms are better equipped for survival.

10.5 ADAPTATIONS FOR THERMOREGULATION

Knowing animals have a tolerance range of temperatures within which they can survive is important. A horse's optimal range for temperature, for example, is 5°C to 25°C. They can survive outside this, within the limits of their tolerance range. However, although they have several adaptations for regulating their body temperature, including sweat glands, most horses do not cope with a lot of exercise in temperatures hotter than about 25°C. To understand how organisms regulate their temperature, it is necessary to understand how heat is transferred. Put simply, if an organism is too hot, it must lose heat. If an organism is too cold, it must gain heat.

Heat transfer

Heat transfer depends on the **temperature gradient** between the internal and external environments. When there is a balance between heat gain and heat loss, the organism is said to be in heat balance,

which is the purpose of thermoregulation. Organisms manage thermoregulation through the interaction of their physiological, structural and behavioural mechanisms.

The mechanisms of heat loss and heat gain are the same: **conduction**, **convection**, **evaporation** and **radiation**.

Conduction is the transfer of heat energy from a hotter object to a cooler object by direct contact. Convection transfers heat when hot air or liquid rises and is replaced by cooler air or water. Convection currents of air remove heat energy from the surface of an organism as they pass over it. Evaporation (in relation to organisms) occurs when a liquid (water or sweat) turns to vapour, cooling the skin. Heat is transferred from the surface of the skin to the water molecules as they evaporate. As the vapour moves off the skin and into the surroundings, the vapour containing the transferred energy carries the heat energy away from the organism. When heat is transferred from an object by infrared waves, it is called radiation. Radiation is the emission of electromagnetic heat waves. Heat radiates from the sun and from dry skin in the same manner. The adaptations, or mechanisms, for heat release (when an organism's internal temperature is elevated above the optimal range) or heat conservation or generation (when an organism's internal temperature falls below the optimal range) help organisms regulate their internal body temperature. The four mechanisms of heat loss and heat gain are illustrated in Figure 10.19.

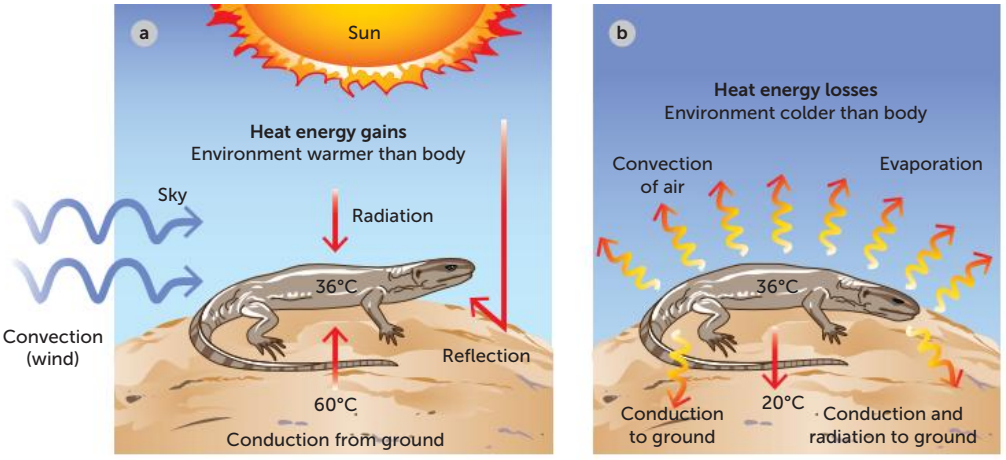

FIGURE 10.19 Heat transfer for a lizard **a** during the day and **b** during sweating and temperature loss at night

Thermoregulation in plants

It is not only animals that can regulate their body temperature. There are some plants that regulate their body temperature. The lotus, *Nelumbo nucifera,* found in the Northern Territory, is able to warm up and regulate its temperature. A bud starts heating up until it reaches approximately 32°C. Its petals start to open, then its temperature will remain constant for 2–4 days, despite fluctuating external temperatures. Just as would happen in a mammal, in the cool of night the plant increases its metabolic heat production, and in the heat of the day **evaporative cooling** comes into play.

Key concept

Thermoregulation is essential for an organism's survival. Heat energy can be lost or gained in the following four ways: conduction, convection, evaporation and radiation.

Thermoregulation in hot environments

For animals living in hot environments, the problem is how to reduce heat gain and increase heat loss. Heat is gained via solar radiation and reflection, ground radiation and conduction, wind currents, and metabolic processes. Both endothermic and ectothermic animals have structural, behavioural and physiological adaptations that allow them to survive in hot environments.

Structural features

Structural features such as dolphin flukes and elephant ears can help cool endotherms when needed. Each half of a dolphin's tail is known as a fluke. When the dolphin is hot, there is increased blood circulation in the tail. The flukes contain many blood vessels just under the skin and so are said to be highly vascularised. They are also very thin, so have a high **surface-area-to-volume ratio**. The heat energy travels via the blood from the core of the body to the tail, where it is released to the cool water by conduction from the blood vessels.

In lieu of sweat glands, elephants, the largest of Earth's terrestrial animals, rely on other physical and behavioural adaptations to keep their massive bodies from overheating. When their surroundings are hot, elephants increase the blood supply to their ears and flap them around to lose body heat. The efficiency of this mechanism is enhanced by the high surface-area-to-volume ratio of their large flat ears. They can achieve a high rate of heat transfer from the veins to the surroundings via radiation.

The red kangaroo has exposed areas of skin on its forelegs to increase evaporative cooling of the blood from this area.

Ectotherms have some structural features that can be used in hot weather. The frilled-neck lizard skin colour can be adjusted to keep its internal temperature within the tolerance range. When desert temperatures rise, their colour becomes lighter, which reflects the heat and keeps the lizard cooler.

In hot climates, fur can insulate animals from radiant heat or hot air around them. For example, the hair on the top of the camel's hump reflects heat.

Alamy Stock Photo/blickwinkel

FIGURE 10.20 African elephants have large flat ears that can be flapped to cool them down (transfer heat out of the body by radiation).

Shutterstock.com/ Matt Cornish

FIGURE 10.21 Frilled-neck lizard of northern Australia

Key adaptation – cooling ears
Read about how elephants have adaptations to keep cool

Behavioural responses

To reduce heat gain, dingoes, birds and rock wallabies normally shelter from high temperatures and reduce their activity, only emerging to feed in the relative cool of dusk and dawn. Avoiding strong sunlight, such as by resting in the shade, reduces heat gain via radiation from the sun and via conduction from hot objects such as rocks.

A number of species of wallabies and kangaroos lick their wrists where the blood vessels form a dense network close to the surface. Even though this means loss of precious water, the evaporation has a cooling effect. The water on the surface of the skin draws heat energy from the body for the change of state from liquid water to vapour. The vapour diffuses into the surrounding air. This is called evaporative cooling.

Other animals such as pigs and hippopotamuses roll in the mud to cool themselves down. As the water in the mud evaporates from their skin, it carries away heat in the same way that sweat does in humans.

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FIGURE 10.22 The behaviour of **a** red kangaroos and **b** dingoes helps them thermoregulate in the heat of Australia. **c** The burrow is a refuge for meerkats because it stays within a narrower temperature range than the air outside, and it is suited to their tolerance range.

Crocodiles are ectotherms that shelter in cool vegetation lining river banks or submerge themselves in the water. They also open their mouths, enabling evaporation from internal surfaces. During cool seasons, they bask in the sunshine to get hot enough to digest their meals.

Echidnas are excellent diggers. Their backward-facing hind legs are unique physical features that push the dirt out of the way. In the burrow, echidnas are protected from harsh weather and can reduce heat gain from the sun's radiation. The cool burrow also enables heat loss via conduction.

Meerkats use burrows for thermoregulation. During the day, when the temperature becomes too hot to continue foraging, they return to the burrow for the shade and cool that it offers. The burrow also assists them at night, when outside temperatures fall below the optimal range, because the temperature in the burrow does not drop as low as the temperature on the surface.

When the daytime heat gets to be too much, elephants enjoy submerging their bodies in water, as well as showering, which entails sucking water in with their versatile, muscular trunks and then spraying themselves. In addition to helping elephants rid their thick skin of parasites, bathing is also an effective way for these enormous animals to reduce their body temperature. Heat from their bodies is transferred to the cooler water via conduction. Thanks to their moisture-retaining wrinkled skin, the cooling effect of a bath or shower continues even after the elephant has left the water.

Physiological adaptations

Many endotherms, such as the red kangaroo, have a number of physiological adaptations for losing heat. When the temperature in the surroundings increases, several physiological responses occur. Nerve impulses stimulate the muscles of certain blood vessel walls to relax, causing the arterioles to dilate (**vasodilation**). This means the blood vessels close to the surface of the skin widen, which allows an increase in blood flow close to the skin's surface, and heat to escape from the blood through the skin via radiation and convection. Vasodilation is a mechanism for cooling the body and counteracts any increase in internal body temperature above the optimal range. A negative feedback model can be drawn to represent the mechanism at work (Figure 10.23).

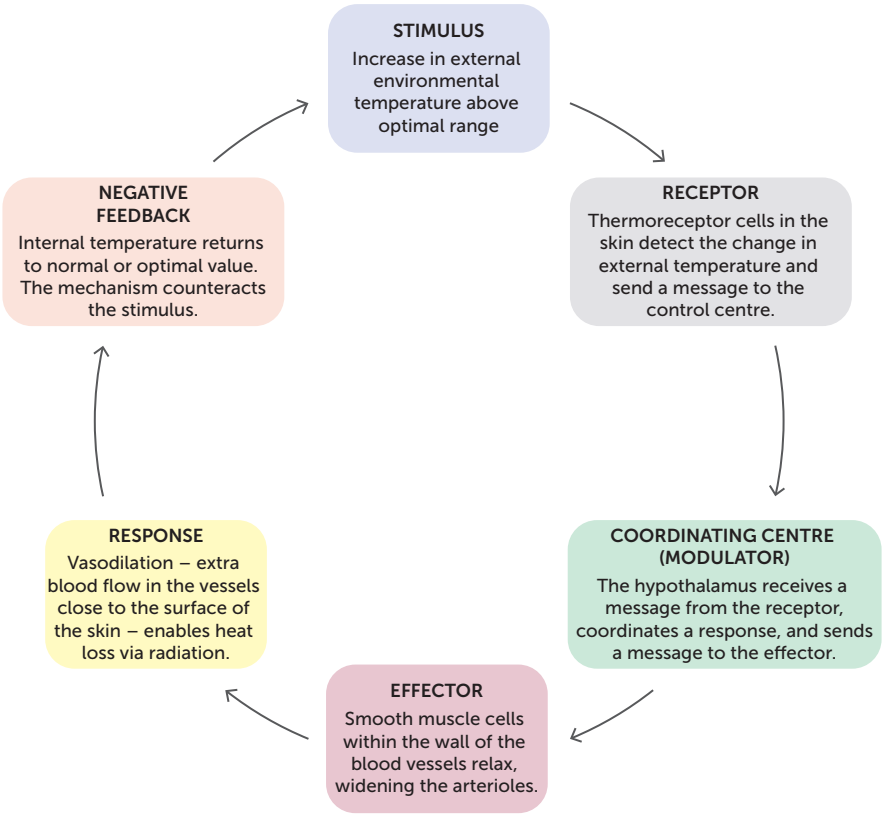

FIGURE 10.23 Negative feedback loop for a physiological response to increased external environmental temperature

Organisms also use **sweating** in the heat – their sweat glands open to release water and salt onto the skin, which evaporates and cools the skin. The evaporated water carries away the heat energy from the body and lowers the internal temperature. This is evaporative cooling. Horses and primates (e.g. humans and monkeys) have large numbers of sweat glands. Other mammals such as kangaroos, pigs, hippopotamuses, dogs and cats do not have many sweat glands and need to employ other mechanisms for temperature control. Many animals, such as dogs, pant to keep cool. **Panting** expels hot air and brings in cooler air, which then helps moisture in the mouth to evaporate quickly, reducing body temperature.

Another response used by endotherms is a decrease in the metabolic rate, which reduces the amount of heat generated within the body. Finally, the muscles attached to hair follicles can be relaxed to flatten the hairs and decrease the layer of air acting as an insulator. This is known as **pilorelaxation**.

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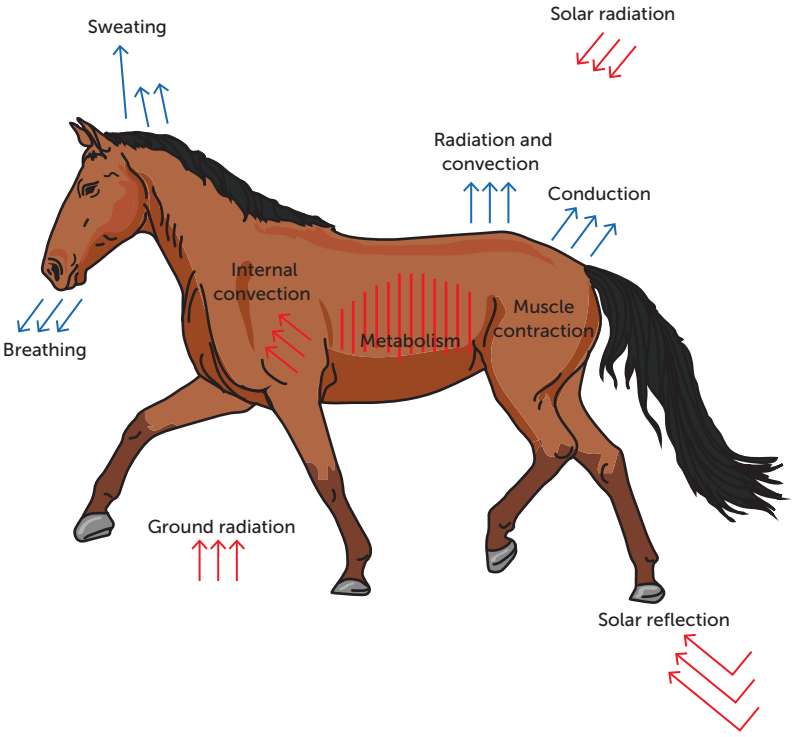

FIGURE 10.24 Heat gains and losses in horses

CASE STUDY

Bilby burrows – important for thermoregulation and hugely important to the ecology of the surrounding habitat

Fun facts about bilbies
Watch this footage of bilbies. Their burrows are vital for temperature regulation.

One of Australia's most iconic marsupials is the greater bilby (*Macrotis lagotis*). Its signature long ears and soft grey fur make the bilby highly recognisable. The greater bilby once occupied around 80% of Australia's terrestrial arid environment. After decades of habitat loss and predation by introduced species such as cats and foxes, bilbies are now only found in a small number of desert regions in WA, Queensland and the Northern Territory.

Currently, the greater bilby's conservation status is rated as vulnerable to extinction. Several conservation strategies have saved the species from the same peril as the lesser bilby, presumed extinct. However, Stuart Dawson, a bilby researcher at Murdoch University, suggests conservation strategies need to

FIGURE 10.25 A bilby emerging from its burrow

be strengthened to match the important role that bilbies play in its ecosystem. The bilby's deep, twisting type burrows serve as a thermoregulatory sanctuary for over 40 other species, such as reptiles. When an animal is absorbing too much heat, it can enter the burrow to avoid some of the heat gain that would occur outside.

Bilbies build their burrow-shelters to reduce heat gain from the radiating sun and to increase heat loss by conduction directly to the cool rock inside the burrow. Temperatures in their outback habitat in WA regularly reach 40°C. Bilbies have other thermoregulatory mechanisms that act in conjunction with their burrowing, such as their ear structure. Bilbies have long, thin, highly vascularised ears that help radiate excess heat.

Scientists have observed bilby burrows by using cameras with motion sensors. A study conducted in northern WA collected footage over a period of 3 years and recorded hundreds of other animals (mammals, birds and reptiles) entering and exiting the burrows.

The inference was made that the animals were using the burrows to stay cool. Bilbies provide an important ecosystem service, because their burrows provide a facility for thermoregulation. If bilby population numbers decrease, the prediction is that there will be a negative impact on other animal species that rely on the cool burrows.

Questions

1. Describe the role of a bilby burrow in thermoregulation.
2. Describe the role of a bilby burrow in the ecology of a habitat.
3. Explain how the bilby's ears can help its thermoregulation.
4. Stuart Dawson would like more funding, research and conservation for bilbies. Construct an argument for his cause.

Bilby conservation breeding program
A successful conservation program is happening at Currumbin Wildlife Sanctuary.

University of Melbourne
See how scientists from Melbourne University have teamed up with Indigenous rangers to protect the greater bilby.

Thermoregulation in cold environments

For animals living in low-temperature environments, the problem is how to reduce heat loss and increase heat conservation. Both endothermic and ectothermic animals have developed structural features, behavioural responses and physiological adaptations to survive in cold environments.

10.1 APPLICATION

Black polar bears

The fur of polar bears looks white, but it is actually colourless; when photographed with film sensitive to ultraviolet light, polar bears appear black. Each strand of hair has a hollow shaft that scatters and reflects visible light, much like ice and snow does. The hollow shaft inspired the hypothesis that the hair acts like an optic fibre, conducting ultraviolet light to the black skin beneath. Experimentation proved this long-standing idea to be wrong; it is now thought that the keratin of the hair absorbs ultraviolet light and that it does not reach the skin.

Shutterstock.com/La Nau de Fotografia

FIGURE 10.26 A polar bear's fur is a thermoregulator.

Structural features

The feathers of mutton-birds and the fur of polar bears aid thermoregulation by trapping an insulating layer of air close to the skin. Insulation reduces the heat flow between an animal's body and its environment. Insulation is found on the surface, in thick fur and feathers, and beneath the surface of the skin, in thick layers of fat (blubber) formed by adipose tissue. Most land mammals and

Homeostasis: blood sugar and temperature
Complete this interactive to observe the changes in skin structure and function at different temperatures.

birds respond to a decrease in external temperature by raising their fur or feathers. This is called **piloerection**, and the process is a physiological mechanism made possible by the structural feature of the thick layer of fur. This traps a thick layer of air, which creates effective insulation, reducing heat loss to the cooler environment. A polar bear generates heat via metabolic processes to keep its internal body temperature stable. Instead of losing this heat to the surroundings via radiation and conduction, it remains warm because it is so well insulated by its thick fur that heat loss is practically nil. The emperor penguin is well insulated by several layers of scale-like feathers, and it takes a strong wind to ruffle them. Although they don't have feathers under their feet, emperor penguins are able to stand on ice for long periods (Figure 10.27). For details of an adaptation that allows this, see Figure 10.28.

Getty Images/Tim Davis/Corbis/VCG

FIGURE 10.27 Emperor penguins have physiological and behavioural adaptations to enable them to survive in the freezing Antarctic temperatures.

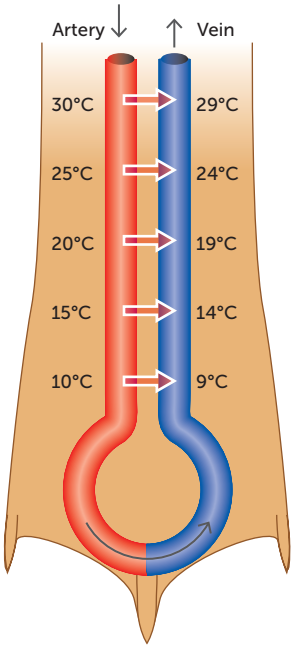

FIGURE 10.28 A model of heat exchange in the foot of an emperor penguin

Variation throughout the year in fur thickness of animals such as French bulldogs and horses is another adaptation that can assist with thermoregulation in challenging environmental conditions.

When temperatures cool, frilled-neck lizards turn darker. Dark colours increase heat absorption from the sun via radiation.

Aquatic birds and mammals can be subjected to very cold environments. Heat loss via conduction from the **extremities** to the aquatic environment is a problem. Fortunately, they have a very effective system of keeping their extremities warm – **countercurrent heat exchange**. Countercurrent heat exchange is the exchange of heat between two fluids flowing in opposite directions in vessels that are in **close proximity** (located close enough for interaction). Heat in the blood travelling in the arteries to the foot or fin warms the blood returning to the body in the adjacent veins. The outgoing blood to the extremity is cooled in the process, but not enough to affect cellular activities. Since the temperature gradient between the extremity and the surroundings is reduced, heat loss is minimised (Figure 10.28). A temperature gradient is produced when two objects in close proximity have different temperatures. The difference in heat energy between the two objects causes heat to travel in one direction, from the hotter object to the cooler object. The countercurrent strategy traps heat in the body core, reducing heat loss in the extremities. Extremities are limbs (arms or legs) that extend away from the core of the body. They contain the outermost regions of an animal's circulatory system and include the hands and feet. They are relatively susceptible to heat loss due to their high surface-area-to-volume ratio.

To understand countercurrent heat exchange, it is necessary to apply your knowledge of the circulatory system. Arteries carry warm blood away from the heart at the core of the body to the extremities. Veins carry cooler blood back to the core from the extremities. Arteries and veins are located adjacent to each other, close enough for heat to be transferred by conduction and radiation. In addition, arteries and veins carry blood that flows in opposite directions, which means there is always a high temperature gradient between them. The 'counter' flow of the blood leads to heat

being exchanged all the way along the length of the exchanger (adjacent artery and vein), increasing the efficiency of the system to a higher rate than if the blood was flowing in the same direction. The result is maximum heat transfer and minimum heat loss to the environment. This structural adaptation can also be found in the flipper of a dolphin.

The shape and size of an organism can help to maintain homeostasis and internal temperature. Large, round animals have a lower surface-area-to-volume ratio than small, thin animals. Adaptations that reduce the surface-area-to-volume ratio reduce heat loss, because there is less surface area per unit volume for heat to transfer through. For example, some bird species in Tasmania tend to be larger than their counterparts on the warmer mainland. The larger size means less surface area is exposed per unit volume, resulting in reduced heat transfer via convection or radiation to the air. Having comparatively small extremities such as ears and legs can reduce the rate of heat loss. The ears and limbs of Arctic foxes are more rounded and smaller than those of their relatives elsewhere (Figure 10.29).

FIGURE 10.29 Ear shape and size differ between **a** the Arctic fox and its relatives, **b** the red fox and **c** the gray fox.

The Australian alpine grasshopper adult male will change the colour of its body surface to help regulate its body temperature in different seasons. At temperatures above 25°C the colour is a bright, greenish blue to reduce heat gain by radiation, and at temperatures below 15°C it is a dull, black colour to increase heat gain from the sun's radiation.

Behavioural responses

Animals can reduce heat loss by minimising the amount of surface area exposed to the surroundings. For example, by huddling together, penguins reduce the group's overall surface-area-to-volume ratio. (They move around within the huddle to prevent any individual from being exposed to the harsh environment for an extended period of time.) Animals can also maximise their heat gain through their behaviour. The freshwater crocodile will choose to bask in the sun when it is cold, to gain heat from the sun via radiation. Additionally, if it is lying on a hot rock, heat will be absorbed by the body via conduction from the rock. When the blue-tongue lizard basks in the sun on a hot rock, it will lie flat to expose more of its body's surface area to the sun and to the rock, maximising the rate of heat transfer via radiation from the sun and conduction from the hot rock.

Frogs and geckos
Investigate WA frogs' and geckos' behaviour and the adaptations they have developed by watching this video on the WA museum website.

Physiological adaptations

Nerve impulses can stimulate the smooth muscle of blood vessel walls to contract, causing the arterioles to constrict (**vasoconstriction**). This makes the blood vessels close to the surface of the skin narrow (decrease in diameter), which results in a decrease in blood flow close to the skin's surface and reduces the amount of heat escaping from the blood through the skin via radiation and convection. Vasoconstriction is a mechanism for reducing heat loss and it counteracts any decrease in internal body temperature below the optimal range. A negative feedback model can be drawn to represent the mechanism at work (Figure 10.30). Vasoconstriction reduces blood flow in peripheral blood vessels, forcing blood towards the core and the vital organs found there, conserving heat.

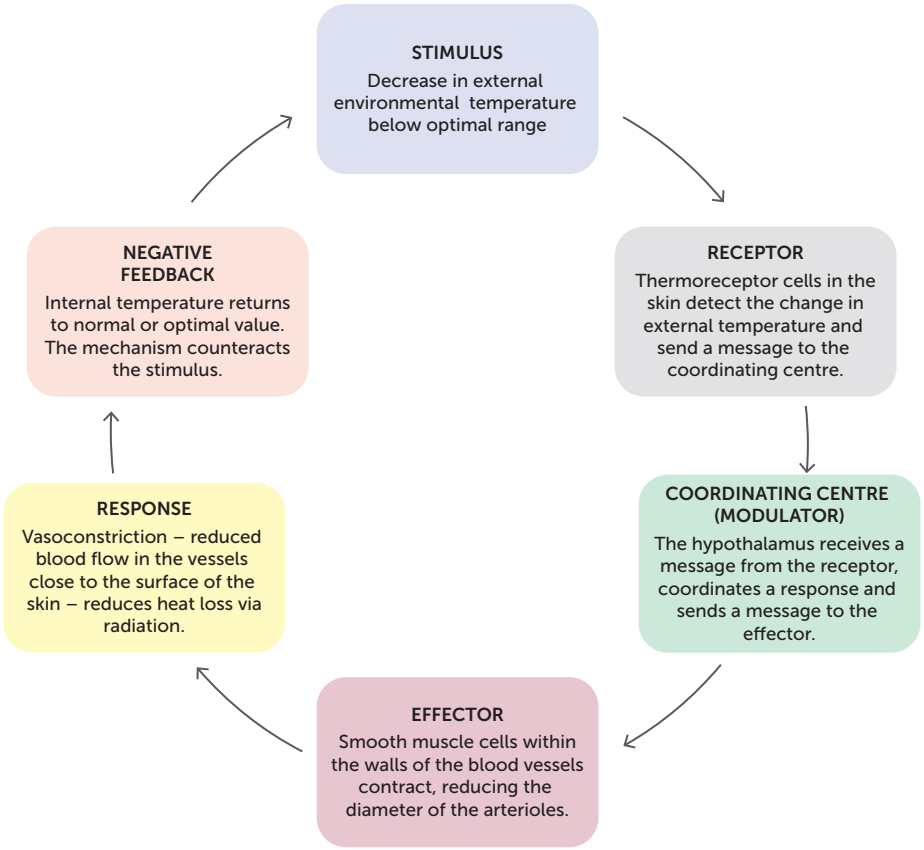

FIGURE 10.30 Negative feedback model for a physiological response to a decrease in external temperature

Metabolic heat production can be used to regulate body temperature. Many animals, especially mammals, use metabolic waste heat as a heat source. When muscles are contracted, most of the energy from the ATP used in muscle actions is wasted energy that translates into heat. Endotherms can vary heat production to suit varying external temperatures.

Shivering is a reflex action activated in many mammals during extremely cold conditions. The high rate of contraction and relaxation of muscles generates heat that can be used to regulate the internal body temperature during the cold period.

Sometimes behaviours and physical features are inadequate for stabilising temperature. At a particular external temperature set point, the metabolic rate of an animal begins to rise, increasing heat output. The external temperature at which the metabolic rate begins to rise is the lower critical temperature, which varies according to species (Figure 10.31). The increase in metabolic activity requires a supply of energy, which for some animals proves difficult if food is scarce.

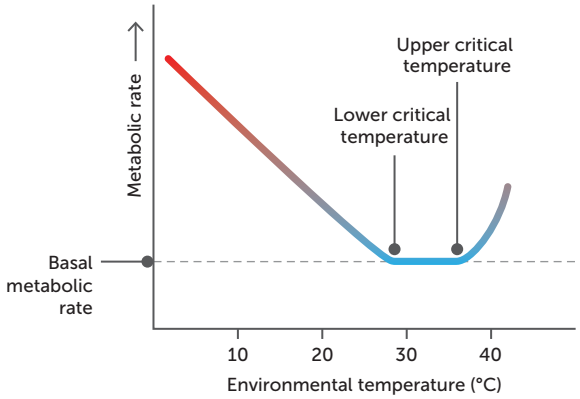

FIGURE 10.31 The effect of environmental temperature on the metabolic rate of a generalised mammal

In very cold conditions, the increase in metabolic rate may be insufficient to maintain body temperature within tolerance limits. A major adaptation that enables animals to save energy, when food is scarce and temperatures are very cold (or very hot), is **torpor**. Torpor is a physiological state of decreased metabolic rate and physical activity.

Many Australian birds and small mammals exhibit a daily torpor. For example, some native bats are active at night for feeding and go into a state of torpor during the day. Torpor reduces energy and water costs for the animal. Another animal that exhibits torpor is the hummingbird, whose internal body temperature can drop significantly during torpor, reducing energy expenditure on metabolic heat production. Other animals in very cold conditions may **hibernate**, spending a longer period in torpor. During hibernation, the metabolic rate falls to a level that just sustains life; the set point is lowered considerably – an excellent mechanism for conserving energy (Figure 10.32). The upper critical temperature is the external temperature at which the body's cooling mechanisms fail.

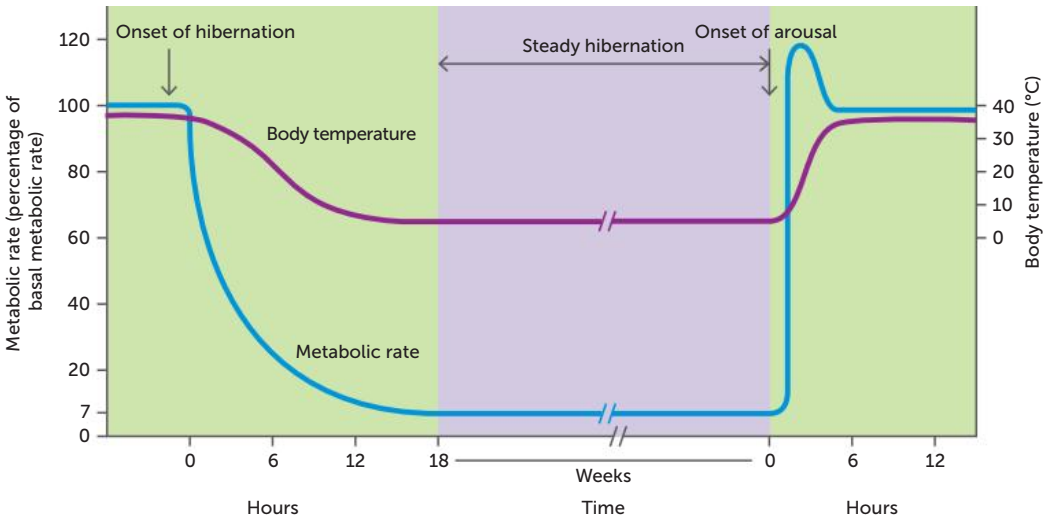

FIGURE 10.32 Metabolic rate and body temperature of a ground squirrel before, during and after hibernation

Another kind of seasonal dormancy is **aestivation** (long-term torpor). This describes what some animals do in very dry conditions, but not necessarily in summer. The garden snail retreats into its shell and seals itself off; some earthworms coil into balls wrapped in mucus that dries out. Lungfish burrow in mud that hardens, and there they remain until the next rainy season, some months later.

TABLE 10.7 Physiological responses to low and high temperatures

EFFECTOR	RESPONSE TO LOW TEMPERATURES	RESPONSE TO HIGH TEMPERATURES
Smooth muscles in arterioles in the skin	Smooth muscles contract, causing vasoconstriction. Less blood is carried from the core to the skin surface. The extremities can turn blue (in lighter skin colours), and the skin feels cold. Less warm blood to the skin surface helps to reduce heat loss to the cool external environment.	Muscles relax, causing vasodilation. More blood is carried from the core to the skin surface. The skin appears red and flushed and feels warm. Heat is lost from the skin's surface by convection and radiation.
Skeletal muscle for shivering Sweat glands for sweating	A reflex action involving the rapid contraction and relaxation of skeletal muscles. This generates heat to increase the body temperature.	Sweat glands secrete sweat onto the skin's surface, from which it evaporates. Heat is absorbed from the skin surface for a change of state from liquid to gas as the sweat evaporates (evaporative cooling).
Muscles attached to hair follicles	Muscles attached to hair follicles contract, raising the hairs and trapping an insulating layer of still, warm air next to the skin. This is not very effective in humans, just causing 'goosebumps' (piloerection)	Muscles attached to the hair follicles relax, lowering the skin hairs and allowing air to circulate over the skin; this encourages cooling by convection and evaporation (pilorelaxation).

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Key concept

Animals and plants have developed adaptations in order to survive in different temperatures. In hot temperatures, thermoregulatory adaptations aim to increase heat loss and reduce heat gain. In cold environments, they aim to reduce heat loss and increase heat gain. The adaptions involved can be behavioural, structural or physiological.

SCIENTIFIC LITERACY

Which animals are best equipped for changes in their environment?

A large number of species currently face extinction. Scientists assert that climate change is a major factor producing this trend. Climate change refers to a significant change in the global climate, as observed in the average and variability of such features as temperature and precipitation, which lasts for a long time, typically decades or longer. Increases in average global temperature as a result of climate change are linked to sea level rises and increasingly extreme weather conditions. These changes are impacting biotic and abiotic factors that enable organisms to survive. Some species are predicted to be more at risk from these changes than others.

Cockroaches are a group of organisms that have an outstanding track record for surviving extreme conditions. They have already survived mass extinction events. Their ability to hold their breath, reproduce fast, eat anything, burrow and tolerate pathogens make them an outstanding competitor for the award of least-threatened species of climate change.

Getty Images/ANNE-CHRISTINE POUJOULAT

FIGURE 10.33 Cockroaches have survived every mass extinction event in history thus far.

Mechanisms for homeostasis have evolved as adaptations. Adaptations can be structural, behavioural and physiological. Just how much capacity species have for adaptation is not known. The effects of climate change and higher temperatures have been observed in our animals in Australia. The effects include coral reef destruction, increased bushfire intensity, marine turtles producing more female than male offspring, and yellow-footed rock wallabies facing starvation from extended drought.

A small (5°C) temperature increase due to climate change would result in drought of such a duration that animals like the rock wallaby would not do well. There would be less food, less habitat, more competition and fewer rock wallabies.

Humans are also temperature sensitive. Slight variations from the optimal internal body temperature of 37°C cause significant effects, as seen in Table 10.8.

The animals that will survive climate change
Read this resource to explore concepts surrounding homeostasis and climate change.

TABLE 10.8 Responses to increased body temperature

BODY TEMPERATURE (°C)	MEDICAL SYMPTOMS
37.7–38.2	Decrease in cognitive skills begins Heat stress Manual skilfulness decreases
38.2–39.2	Increase in judgement error Decrease in tracking skills Potential heat exhaustion
39.2–39.6	Functional limit of physical tasks is reached Likely heat exhaustion Potential heat stroke
>39.6	Probable heat stroke

Questions

1. From the data in Table 10.8, list some symptoms experienced by humans when internal body temperature exceeds normal by 2°C.
2. Using your knowledge of enzymes explain some of the symptoms.
3. Name an animal that can survive extreme changes in environmental conditions.
4. Explain why your named animal has a higher chance of survival than the rock wallaby.

Question set 10.5

REMEMBERING

1. Draw a diagram of an Australian reptile and illustrate each of the following methods of heat transfer used when the reptile is experiencing a cold day and employing behaviours that help increase body temperature:
   - **a** conduction
   - **b** convection
   - **c** radiation.
2. Copy and complete Table 10.9 to compare a mammal's physiological responses to hot and cold temperatures.

TABLE 10.9 Simple stimulus–response mechanisms involved in thermoregulation

STIMULUS	PHYSIOLOGICAL RESPONSE	EFFECT
Increase in temperature		More heat lost through radiation
	Hairs flatten on skin, trapping less air	
Decrease in temperature	Constriction of blood vessels on the skin	
		Less heat loss through conduction
	Shivering	

3. Explain how heat balance is achieved.

UNDERSTANDING

4. What is vasodilation? Explain how it helps to maintain internal temperature.
5. Explain the difference between hibernation and torpor.
6. Explain how the shape of an organism can aid thermoregulation.
7. Explain how countercurrent heat exchange can help to maintain internal temperature.

CREATING

8. Construct a negative feedback model to demonstrate thermoregulation in mammals experiencing cold weather through the mechanism of shivering.

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CHAPTER 10 ACTIVITY

10.1 ACTIVITY

Water balance in animals

Aim

To study water regulation in *Paramecium* and humans

What to do

Read the following information and answer the set of questions.

A culture of *Paramecium* was placed on a thin layer of petroleum jelly on a microscope slide. A coverslip was added and the cells were observed.

The anterior and posterior contractile vacuoles were located, and the time between contractions of the *Paramecium* culture was noted. The *Paramecium* culture was exposed to differing concentrations of sucrose from 0 to 50 mmol L^{-1}. The relationship between the time between contractions and the concentration of sucrose in the surrounding solution was displayed in a graph (Figure 10.34).

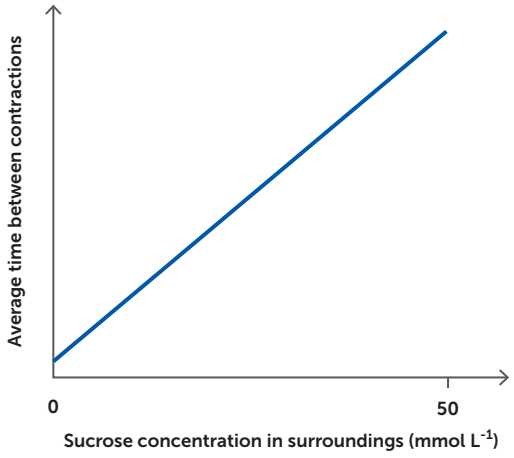

FIGURE 10.34 The relationship between the time between vacuole contractions and the concentration of sucrose in the surrounding solution

Analysis of results

1. When a *Paramecium* lives in its normal freshwater environment, it is subjected to a continuous influx of water. Explain why.
2. Describe what happens to the time between vacuole contractions as the concentration of the surrounding sucrose solution increases.
3. How would the rate of water expulsion from *Paramecium* change as the osmotic pressure of the surroundings increased (solutes became more dilute)?
4. How could you tell when *Paramecium* was in an isotonic solution?
5. Using information from the experiment, explain how the contractile vacuoles in *Paramecium* enable the cell to maintain a steady internal solute concentration.
6. Would this process of osmoregulation continue if the energy supply of the cell was cut off? Explain.

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CHAPTER 10 SUMMARY

Chapter 10 Activity sheet

- Homeostasis is the maintenance of a relatively stable internal environment within a small tolerance range, despite changes in the external environment.
- Multicellular organisms contain receptors that are highly specialised for receiving signals from the external and internal environments.
- The nervous system provides a fast response to stimuli transmitted via sensory neurons in the PNS to the CNS and back via motor neurons in the PNS to effectors (muscles and glands).
- The endocrine system provides a relatively slower, long-lasting response involving the release of hormones. It is mainly controlled by the pituitary gland.
- The nervous and endocrine systems react to changes in stimuli and respond to them (the stimulus–response model).
- Negative feedback counteracts a change in a stimulus to maintain internal pH, water and solute concentrations within narrow limits.
- Positive feedback reinforces a change in a stimulus and is seen in developmental processes. Positive feedback can be harmful to homeostasis.
- Organisms must keep inorganic and organic materials, pressure and temperature within narrow limits for survival. These limits are known as tolerance limits. Each organism has an optimal range within which it functions best; outside this range is the zone of physiological stress, within which it can survive. Outside that range, in the zone of intolerance, it cannot survive.
- Adaptations allow organisms to maintain homeostasis within their tolerance limits. They include physiological processes, structural adaptations and behavioural functions.
- The main factors regulated within the tolerance limits are temperature, nitrogenous waste, water, salts and gases.
- Thermoregulation is essential for preventing hyperthermia and hypothermia. Vertebrates have physiological mechanisms, structural features and behavioural strategies that aid the regulation of core body temperature.
- Organisms can be classified as endotherms or ectotherms.
- Heat energy can be transferred in four ways: conduction, convection, evaporation and radiation.
- Mechanisms for thermoregulation in a hot environment include sweating, vasodilation, panting, large round body shape, and increased breathing rate for further evaporative cooling.
- Mechanisms for thermoregulation in a cold environment include shivering, adjusting to a higher metabolic rate, vasoconstriction, countercurrent heat exchange, torpor and piloerection.

CHAPTER 10 GLOSSARY

Adaptation An evolved structural, physiological or behavioural characteristic of an organism that increases its chances of survival and reproduction in a particular environment

Aestivation Long-term torpor; dormancy that occurs in some animals during periods of drought; reduced metabolic rate

Ammonia The direct product of the breakdown of protein or nucleic acids; it is extremely toxic and highly soluble in water

Axon The extension from a neuron cell body along which an electrical impulse can travel towards a target cell

Behavioural adaptation An adaptation that relates to how an organism acts in response to a stimulus

Catabolic reaction A chemical (metabolic) reaction whereby bonds in molecules are broken, releasing energy

Chemoreceptor A sensory cell or organ that detects chemical stimuli

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Close proximity Located close enough for interaction

Conduction The transfer of heat energy from a relatively hot object to a relatively cool object by direct contact

Convection The transfer of heat by means of rising currents of warm air or water

Coordinating centre (modulator) A tissue or organ that receives messages from receptors (via sensory neurons) and coordinates a response, then sends the information to an effector via motor neurons; usually the hypothalamus

Countercurrent heat exchange The exchange of heat between two fluids flowing in opposite directions in vessels that are in close proximity

Ectotherm An animal whose body temperature is determined by the external environment. Ectotherms rely on structures and behaviours for thermoregulation. Ectotherms may obtain heat from the sun or from objects in their surroundings, which means their body temperature fluctuates with that of the external environment

Effector A muscle or gland that receives a message from the control centre that a change in a stimulus has occurred, then carries out a response

Endocrine system The bodily system responsible for the production and secretion of hormones, which are released into the bloodstream to act on specific target cells and organs

Endotherm An animal that uses metabolic processes to generate its own heat to maintain its internal temperature within the tolerance range. Endotherms also have a range of adaptations that serve as mechanisms for controlling heat gain or loss. Within tolerance limits, animals such as birds and mammals can maintain a stable internal temperature independent of external temperature fluctuations

Enzyme A reusable, biological catalyst that lowers the activation energy of chemical reactions, making them proceed faster; it is a protein that is sensitive to factors such as temperature and pH

Estuary A transitional region in which fresh water from a river meets salt water from the sea

Evaporation The process in which liquid water changes to water vapour through heating

Evaporative cooling A mechanism of heat transfer from an organism to its surroundings. Water on the surface of the skin draws heat energy from the body for the change of state from liquid water to vapour. The vapour diffuses into the surrounding air, taking heat away from the body and cooling the body down

Extremities The ends of limbs (arms or legs) that extend away from the core of the body; extremities contain the outermost points of an animal's circulatory system and can include feet and hands

Feedback mechanism A mechanism in which the output or response affects the input or stimulus

Hibernate A period of dormancy over a long period of cold conditions

Homeostasis The processes involved in maintaining a constant internal environment, within tolerance limits, despite changes in the internal and external environment

Hyperthermia A state in which an organism's internal temperature rises above the upper tolerance limit

Hypertonic At a higher concentration than another solution. When a cell is surrounded by a hypertonic solution, water moves out of the cell via osmosis to dilute the surroundings, so the cell shrinks

Hypothalamus A small region of the brain that plays a major role in detecting and coordinating the response to a change in e.g. temperature or water content in the blood

Hypothermia A state in which an organism's internal temperature drops below the lower tolerance limit

Hypotonic At a lower concentration than another solution. When a cell is surrounded by a hypotonic solution, water moves into the cell via osmosis to dilute the cell, so the cell swells. (Animal cells, which have no cell wall, sometimes burst.)

Insulation Any structure that reduces the heat flow between an animal's body and its environment, including thick fur or feathers,

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and beneath the surface of the skin, thick layers of fat (blubber) formed by adipose tissue

Interconnecting neuron Located in the central nervous system, a nerve cell that transfers signals from sensory neurons to motor neurons

Interstitial fluid The fluid that lies in the spaces between cells; also known as tissue fluid

Isotonic At the same concentration as another solution. If a cell and its surrounding solution are isotonic, there is no net movement of water between them and the cell maintains a constant volume

Mechanoreceptor A sensory cell or organ that responds to mechanical stimuli

Metabolism The sum of all the chemical reactions occurring within an organism to maintain life; it includes reactions enabling an organism's growth, homeostasis and reproduction

Motor neuron A nerve that transmits impulses from the central nervous system to an effector

Myelin sheath The fatty layer surrounding and insulating the axons of many neurons; it increases the speed at which electrical impulses travel along the nerve cell

Negative feedback When a change in a variable (stimulus) occurs, it is a response that counteracts the change and returns the variable back to its normal (optimal) value

Nervous system The network of nerve cells and fibres that transmits nerve impulses to provide communication between parts of the body

Nitrogenous waste The nitrogen-containing metabolic waste products of the breakdown of proteins and nucleic acids. Initially, ammonia (which is highly toxic) is formed. Many animals convert ammonia into a less toxic form – either urea or uric acid

Optimal range The narrower range, within an organism's tolerance range for a particular factor, at which the organism functions best

Osmotic pressure The force of pressure that results when there is a difference in solute concentration across a membrane

Pain receptor A sensory cell or organ that detects pain signals

Panting A method of cooling through evaporation of water from internal body surfaces via exhalation

pH A measure of how acidic or alkaline (basic) an aqueous solution is. It is a measure of the hydrogen ion concentration. Solutions with a pH of 7 are neutral. If the pH is above 7, the solution is alkaline; if the pH is below 7 the solution is acidic.

Photoreceptor A sensory cell or organ that detects light signals

Phototropism A plant's hormonal response to light, whereby auxin accumulates on the darker side of the plant to stimulate cell elongation, bending the plant towards the light to increase its ability to photosynthesise

Physiological process A functional process that is performed by organisms to maintain life

Physiological stress Stress caused when an organism experiences conditions outside its optimal range

Piloerection A physiological mechanism made possible by the structural feature of a layer of fur; the muscles attached to hair follicles contract, so that the hairs stand up, trapping a layer of air that can provide insulation, reducing heat loss to a cooler external environment

Pilorelaxation A physiological mechanism involving muscles attached to hair follicles relaxing to flatten hairs and decrease the layer of air acting as an insulator. This enables more heat loss and cools the body

Pituitary gland Coordinates the endocrine system activities; it is the master endocrine gland because it produces hormones that affect the production of other hormones

Plasmolysis The state of a plant cell in which the cell membrane has pulled away from the cell wall due to water moving out of the cell

Positive feedback When a change in a variable (stimulus) occurs, it is a response that amplifies (further increases) the change

Radiation The transfer of heat from a hot object by infrared waves

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Receptor A cell or tissue that detects a stimulus (change in the environment); the receptor may be internal or external

Response The action of the effector that counteracts or amplifies the stimulus; the change made by the effector

Sensory neuron A nerve that transmits nerve impulses from a receptor towards the central nervous system

Set point The optimal value for an internal variable such as temperature or Ca^{2+} concentration

Shivering A reflex action activated in many mammals during extreme cold conditions, in which a high rate of contraction and relaxation of muscles generates heat that can be used to regulate the internal body temperature

Solvent A substance in which another substance (known as a solute) dissolves

Stimulus (plural stimuli) A change in one of the internal or external environmental factors; it can be detected by a receptor and involves a deviation from the normal or optimal value

Stimulus–response model The two stages of homeostatic regulation. The stimulus (stage one) is the detection of a change from the stable state; stage two is the response to the stimulus, which can be described as counteracting the change (negative feedback) or amplifying the change (positive feedback). A stimulus–response model can be used for homeostasis

Structural features Physical features that usually have a function; they include cellular structure and the size and shape of an organism

Substrate A substance on which an organism acts

Surface-area-to-volume ratio The ratio of the surface area of a structure or organism to its internal volume

Sweating A process in which sweat glands open to release water and salt onto the skin; the sweat then evaporates and cools the skin

Temperature gradient Produced when two objects in close proximity have different temperatures due to different amounts of heat energy; this causes heat to travel from the hotter object to the colder object

Thermoreceptor A sensory cell or organ that detects heat or cold

Tolerance range The range of a factor within which an organism can function and reproduce; if factors go outside of this range, it may be fatal for the organism

Torpor A physiological state of decreased metabolic rate and physical activity

Urea A nitrogenous waste formed from the breakdown of proteins and nucleic acids (nitrogen-containing compounds); it is a less toxic form than ammonia, but the conversion from ammonia to urea requires energy; it is moderately soluble in water

Uric acid A nitrogenous waste formed from the breakdown of proteins and nucleic acids (nitrogen-containing compounds); it is the least toxic form, but the conversion from ammonia to uric acid requires a large amount of energy; it is in the form of a semi-solid paste and is insoluble in water

Vasoconstriction A physiological mechanism in which blood vessels contract to reduce blood flow and therefore heat loss from the surface of the body

Vasodilation Dilation (widening) of blood vessels, particularly arterioles

Zone of intolerance The zone that is outside the tolerance range for survival

Zone of physiological stress The zone that is outside the optimal range, but inside the tolerance range; it is not optimal, but within it survival is possible

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CHAPTER 10 REVIEW QUESTIONS

Remembering

1 Define homeostasis.

2 Using an example, explain the principle of positive feedback.

Understanding

3 Explain why some ectotherms bask in the sun.

4 When a cold reptile lies on a warm rock, it spreads its whole body out. Explain the purpose of this behaviour.

5 Draw a table to summarise examples of the structural, physiological and behavioural adaptations a mammal can use to regulate temperature.

6 Referring to Figure 10.29 (page 359), account for the differences shown in the size and shape of the ears of different species of fox.

7 Kangaroos do not sweat when they rest. Instead, they breathe at a higher rate. Explain how breathing may assist in lowering a kangaroo's body temperature.

Applying

8 Homeostasis maintains the constant internal environment necessary for survival. One factor it regulates is blood calcium concentration. Name another salt that is under homeostatic control and explain why it must be regulated.

9 What strategies do animals employ if they are unable to meet their energy needs?

10 Name an animal that lives in conditions of either extreme cold or extreme heat. Draw a concept map to summarise the structural, physiological and behavioural adaptations it has available to regulate its temperature.

11 Explain the significance of receptors failing.

Analysing

12 Figure 10.35 shows a mechanism known as countercurrent heat exchange. Copy and annotate the diagram to show the principles of countercurrent heat exchange.

13 Figure 10.36 shows the relationship between the environmental temperature and the metabolic rate of various animals. The basal metabolic rate for each animal is given a value of 100%. Any increase in metabolic rate is in relation to this value.

- **a** At what external temperature does the metabolic rate of the Eskimo dog pup begin to increase?
- **b** At what external temperature does the metabolic rate of the sloth begin to increase?
- **c** The gradients of the lines of the graph indicate the rate of the increase in metabolic rate. Which animal, the ground squirrel or the polar bear cub, shows the greater rate of increase in metabolic rate?
- **d** Analyse the information in the figure and compare species living in arctic conditions with species living in tropical conditions.

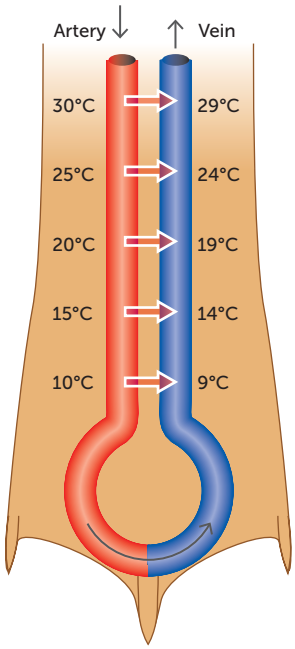

FIGURE 10.35 Model of countercurrent heat exchange in the foot of an emperor penguin

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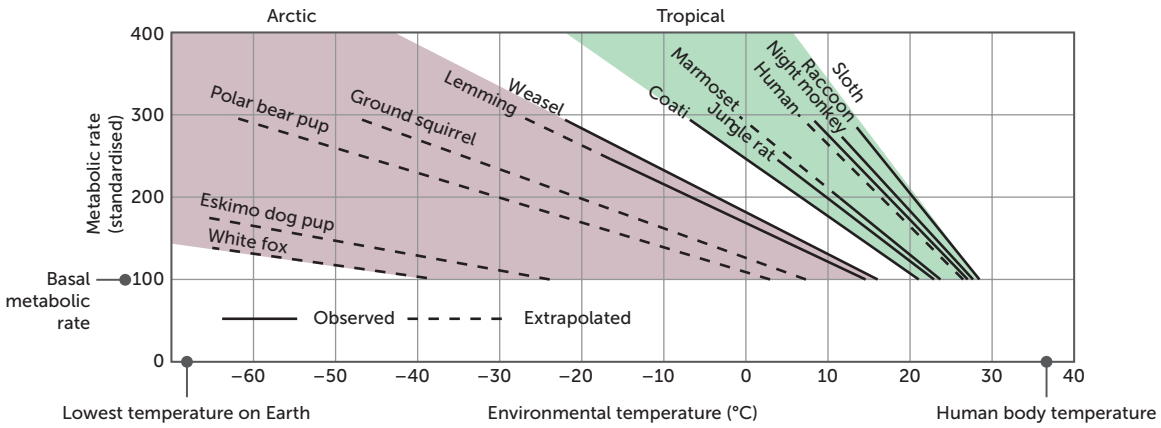

FIGURE 10.36 The relationship between environmental temperature and metabolic rate

Evaluating

14 The removal of waste products from the interstitial fluid is essential in maintaining optimal metabolic function. Explain this statement.

Creating

15 Design a new species of ectotherm suited to living in a hot environment. Describe its structural and behavioural adaptations for thermoregulation.

PRACTICE EXAM QUESTIONS

1 Penguins have an inner layer of soft feathers that trap air close to the body. This reduces heat loss due to:
- **A** conduction
- **B** convection
- **C** evaporation
- **D** radiation.

[Q14 2019 SCSA]

2 Penguins also have a thick layer of fat. This reduces heat loss due to:
- **A** conduction
- **B** convection
- **C** evaporation
- **D** radiation.

[Q15 2019 SCSA]

3 A behavioural adaptation that some animals use to regulate their body temperature in a hot environment is:
- **A** panting
- **B** burrowing
- **C** vasodilation
- **D** vasoconstriction.

[Q18 2019 SCSA]

4 A terrestrial bird will lose most water by:
- **A** breathing
- **B** feeding
- **C** sweating
- **D** urinating.

[Q8 2018 SCSA]

Question 5 relates to the information below.

A biologist measured the internal temperatures of six healthy grasshoppers from the same species in their natural environment. The measurements were 32.8, 37.8, 35.0, 37.0, 33.0 and 36.7°C. The environmental temperature at the time was 36°C.

5 The median body temperature of the grasshoppers in °C was:
- **A** 35.0
- **B** 35.4
- **C** 35.9
- **D** 36.0.

[Q11 2018 SCSA]

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6 A lizard lying on a rock that is warmer than the body of the lizard will:

A lose heat to the rock by convection
B lose heat to the rock by conduction
C gain heat from the rock by convection
D gain heat from the rock by conduction.

[Q20 2018 SCSA]

7 **a** Outline the role of the effector in homeostasis. (2 marks)

b Outline the role of the receptor in homeostasis. (2 marks)

c State the defining feature of a negative feedback loop. (1 mark)

[Q33a 2019 SCSA]

8 A stimulus–response model consists of several parts, which are represented by the boxes in the diagram below. The part represented by box (v) has been labelled. Complete the diagram by placing the correct labels for the different parts of the model in boxes (i) to (iv). (4 marks)

[Q31a 2018 SCSA].

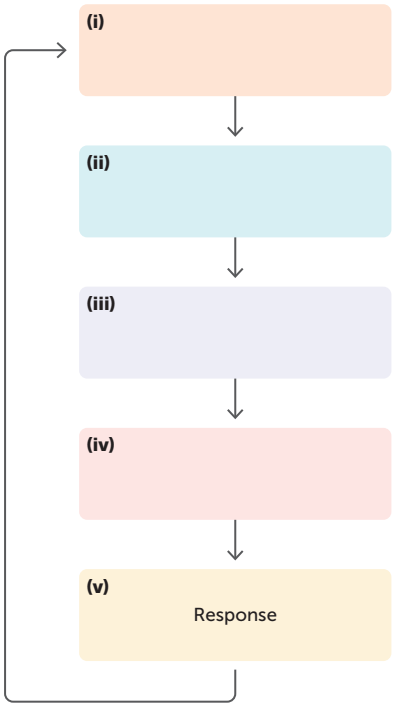

FIGURE 10.37 Negative feedback model

9 Rabbits have the ability to control the amount of blood flow to their ears. Explain how this can help them to thermoregulate. (4 marks)

[Q35d 2017 SCSA]

10 Compare the methods that endotherms and ectotherms use to regulate their internal body temperature and discuss the costs and benefits of endothermy to individuals. (10 marks)

[Q38 2019 SCSA]

11 The Arctic fox (Figure 10.29a, page 359) lives in the Arctic tundra, which is one of the coldest environments on Earth. Discuss one structural feature and one physiological process that enables mammals living in cold environments to maintain a constant core body temperature. Identify clearly in your answer which is the structural feature and which is the physiological process. (10 marks)

[Q38a 2018 SCSA]

12 Describe in general terms how an organism maintains its internal environment within its tolerance limits. (10 marks)

[Q38 2017 SCSA]

11 REGULATION OF WATER, SALTS AND GASES

CHAPTER 11 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Can you explain the difference between urea and urine?
2 Why do some mammals have longer loops of Henle than other mammals?
3 How does a cactus survive in the desert?

SCIENCE UNDERSTANDING

» the type of nitrogenous waste produced by different vertebrate groups can be related to the availability of water in the environment
» animals have a variety of behavioural, physiological and structural adaptations to maintain water and salt balance in terrestrial and aquatic environments
» to maintain water balance and allow for gas exchange, xerophytes and halophytes have a variety of structural and physiological adaptations

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

Getty Images/Max shen

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11.1 WATER: ESSENTIAL TO LIFE

Water is the universal **solvent** and is essential to life. A solvent is a substance in which another substance (known as a **solute**) dissolves. Most salts and minerals in organisms are dissolved and broken into ions by water. Organisms therefore contain an aqueous solution of ions, such as sodium and chlorine ions, ready for metabolic processes.

Metabolic reactions occur in a solution composed mainly of water. When the water content is too high or too low, metabolic reactions slow down, because the reactants travel too slowly to their reaction sites. Blood plasma, which transports metabolic products, is approximately 90% water. Blood not only supplies nutrients to cells, but also transports waste products away from cells for removal from the organism. Typically, the main waste products that require removal are carbon dioxide, via the lungs, and nitrogenous compounds, via the kidneys.

Water balance requires continual homeostatic control, or **osmoregulation**. Osmoregulation is the active regulation of the organism's water content; that is, it maintains the fluid balance (water gain and loss) and the concentration of electrolytes (ionic solutes, or salts in solution) and other solutes so the fluids don't become too diluted or too concentrated. If the supply of water does not replace what is being lost, the relative concentrations of solutes and water in tissue fluids become difficult to regulate. Physiological functions are then affected. A loss in blood volume results in a blood pressure drop, toxic wastes not being excreted effectively and enzyme function being affected. Severe dehydration can lead to death. In plants, loss of water can mean collapse of shoot systems and interference with cellular functioning. Concentrations of water and solutes need to be kept within narrow tolerance limits. In animals, ions such as sodium and calcium need to be maintained at concentrations that permit the normal activity of muscles and neurons.

Water is constantly lost through evaporation (especially through evaporative cooling mechanisms such as sweating, panting and rapid breathing) and urination. Water can be replaced by drinking and eating; however, homeostatic mechanisms are also employed by many animals to regulate their water content. Changes in the water level in the blood are detected by receptor cells in the **hypothalamus** (called **osmoreceptors**). The hypothalamus is a small region of the brain that plays a major role in detecting changes in the blood. In addition to detecting changes, the hypothalamus acts as a **coordinating centre (modulator)**, receiving information and coordinating a response. The hypothalamus alters the kidney membrane permeability to adjust the concentration of the urine, allowing wastes in the form of solutes to be excreted while conserving water.

A cell into which water has diffused so that the walls are stretched and the cell is fairly rigid is described as **turgid**. Water can move into and out of cells and organisms via **osmosis**. Osmosis is the passive diffusion of water across a membrane in response to a concentration gradient (osmotic pressure) caused by an imbalance of molecules on either side of the membrane. The concentration of a cell's watery surroundings relative to that of the cellular contents can be:

1. **isotonic**: when the surroundings are of equal concentration to the cellular contents and there is no net movement of water. Water may move in and out via diffusion, but the total input and output is equal, which is known as zero net movement. The cell maintains a constant shape, since the water volume is constant. This is the optimal state.
2. **hypertonic**: when the surroundings are more concentrated than the environment. When solutions separated by a semipermeable barrier are of different concentrations, water will move across the barrier via osmosis in order to equalise the concentrations. Thus, in the case of hypertonic solutions, water moves out of the cell in order to dilute the outside concentration, bringing it closer to the cellular concentration. Since water is leaving the cell, the cell loses turgidity and shrivels up.
3. **hypotonic**: when the surroundings are less concentrated than the cellular contents. This causes water to move into the cell via osmosis, making the cell contents less concentrated and making the surroundings more concentrated. As a result, the cell volume increases and the cell swells up, possibly bursting if too much water enters.

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Key concept

Osmoregulation refers to the gain and loss of water and the concentration of solutes. Osmosis is the passive movement of water across a membrane. Solutions can be isotonic (of equal concentration, water movement is equal or net zero), hypertonic (more concentrated outside the membrane, water moves out) or hypotonic (less concentrated outside the membrane, water moves in).

The kidneys

Formation of urine
This website contains an animated tutorial and quiz summarising the structures and function of the kidney.

Kidneys are essential organs involved in maintaining a constant internal environment. They play an important role in osmoregulation. Their osmoregulatory function includes:

1. removal of **nitrogenous wastes**
2. regulation of water concentration in the blood
3. maintaining ion levels in the blood.

The cortex and medulla make up two of the main layers in a kidney and are composed of individual filtering units known as **nephrons**, which filter the blood in order to regulate chemical concentrations and produce urine. At one end of each nephron, in the cortex (outer layer) of the kidney, are cup-shaped structures called **Bowman's capsules**. Each one surrounds a group of capillaries called a **glomerulus**. Blood travels from the renal arteries into the glomerulus of each nephron. At the glomerulus, plasma is forced out through the walls of the glomerulus, then in through the outer layers of the Bowman's capsule to its interior, being filtered in the process. After entering the capsule, the filtered fluid (**filtrate**) flows along the proximal convoluted tubule to the loop of Henle, and then to the distal convoluted tubule and the collecting ducts, finally flowing into the ureter. Each of the various components of the nephrons are selectively permeable to different molecules, and enable the complex regulation of water and ion concentrations in the body. Renal arteries carry blood to the kidney and renal veins carry blood away from the kidney. The glomerulus is the site in the nephron where fluid and solutes are filtered out of the blood to form a glomerular filtrate. The process is called **filtration**. The proximal and distal tubules, the loop of Henle, and the collecting ducts are sites for the **reabsorption** of water and ions. Reabsorption is the process of water, ions and other substances in the filtrate being absorbed back into the blood. Together, filtration and reabsorption in the mammalian nephron regulate body fluid concentrations.

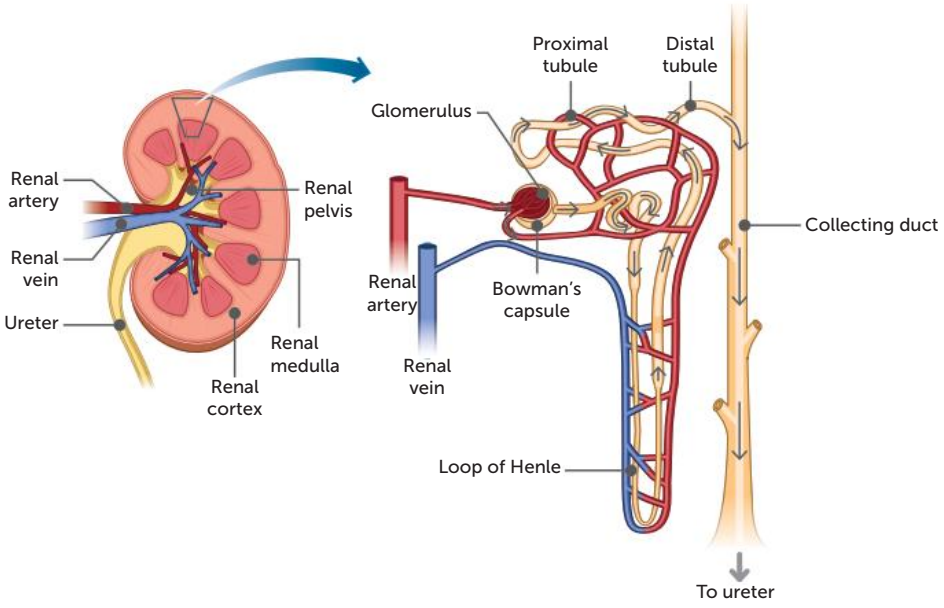

FIGURE 11.1 Structures of the human kidney and nephron substances that are reabsorbed (blue represents water; grey represents salts and other substances)

Question set 11.1

REMEMBERING

1 Why is water essential to life?
2 Name and describe the three types of solution that may surround a cell.
3 State three main functions of the kidney.

UNDERSTANDING

4 Differentiate between filtration and reabsorption in nephrons.
5 a Explain the difference between solute and solvent.
b Why might water be known as the universal solvent?

APPLYING

6 Draw a diagram of a nephron and label the main parts.

11.2 NITROGENOUS WASTES

Nitrogenous wastes
To further your knowledge and understanding of nitrogenous wastes, visit this website.

Excretion is the removal of nitrogenous wastes. In mammals, the nitrogenous waste **urea** is removed as part of the urine. The elimination of nitrogenous wastes (formed from the breakdown of protein molecules and nucleic acids) is essential. One such nitrogenous waste is **ammonia**, which is extremely toxic. A build-up of ammonia in cells can affect their pH, making them more basic, which can denature enzymes and compromise their function. This, in turn, can reduce metabolic activity. The toxicity can be lessened if the ammonia can be diluted in water, but that depends on the availability of water. Various organisms have different ways of coping with this waste product. Some animals excrete ammonia directly, while many others expend energy to convert ammonia to a less toxic form, urea or **uric acid**, before excretion.

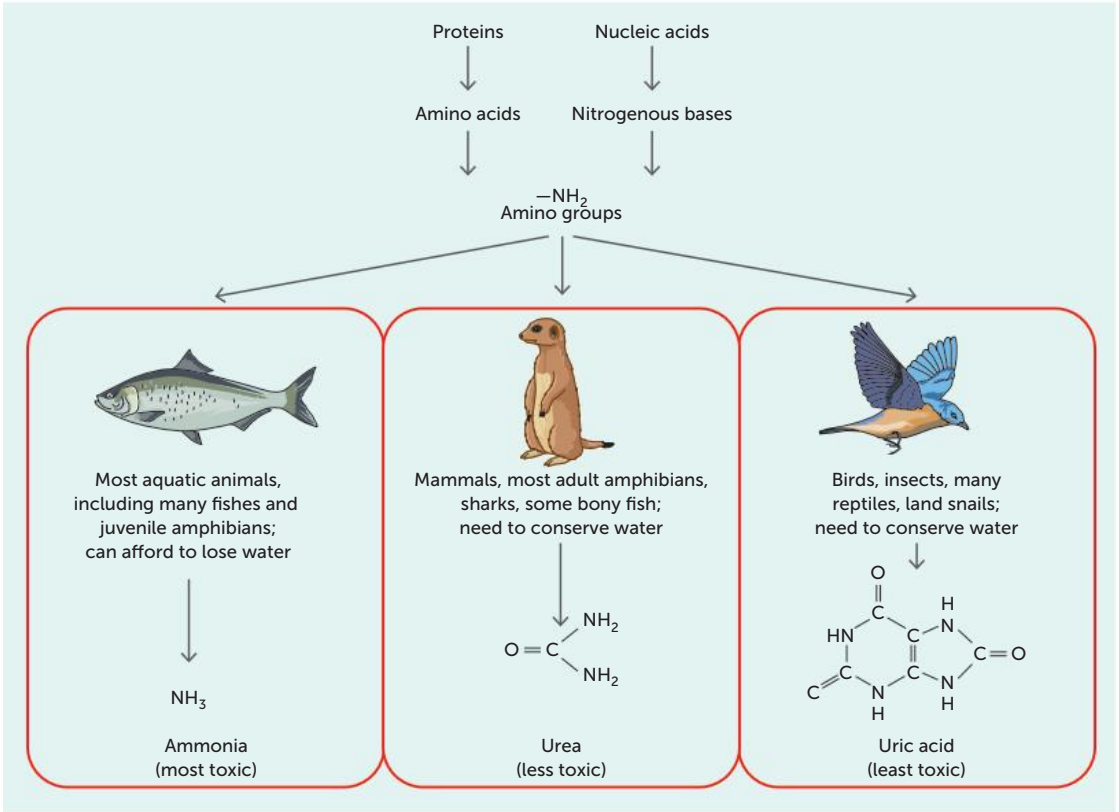

FIGURE 11.2 Three types of nitrogenous wastes and where they occur, which relates to water availability in the organism's environment

Freshwater fish produce abundant amounts of dilute urine containing ammonia. It is excreted quickly and continuously. On the other hand, marine fish and terrestrial (land) mammals must quickly convert the ammonia to a less toxic substance, urea. It is then released as concentrated urine, reducing water loss from the body. Other organisms, such as reptiles and birds, produce uric acid, which is the least toxic form of nitrogenous waste and contains very little water.

Natural selection has resulted in organisms that excrete the type and amount of nitrogenous waste that works best for the availability of water in the environment in which they evolved. Evidence of this is seen in turtles. Terrestrial turtles excrete mainly uric acid, in contrast to aquatic turtles, which excrete both urea and ammonia. In many frog species, the tadpoles excrete nitrogenous waste as ammonia, whereas the adult frogs excrete urea. This is because tadpoles live in water and are able to dilute the highly toxic ammonia. A high volume of water output is essential in order to prevent death from ammonia toxicity in the tadpoles. Adult frogs live on land and are usually able to access smaller amounts of water than tadpoles. With less available water, adult frogs excrete urea. This has the added benefit of saving the frog water, but it takes energy to convert ammonia to urea.

This is not observed in aquatic mammals. Even though whales and dolphins live in an environment with copious amounts of water for diluting ammonia, they evolved from terrestrial mammals, and features such as urinary systems were inherited from their ancestors. Consequently, whales and dolphins excrete urea in their urine.

TABLE 11.1 Comparison of nitrogenous wastes in various vertebrate groups

NITROGENOUS WASTE	VERTEBRATE GROUPS	SOLUBILITY AND ENVIRONMENTAL WATER REQUIREMENT	TOXICITY	ENERGY COSTS
Ammonia	Fish Juvenile amphibians Aquatic reptiles	Ammonia is highly soluble. In some invertebrates it can dissolve in water and pass directly through body surfaces. An environment high in water is required, such as in aquatic environments, to dilute the ammonia.	Very high Requires dilution with water Highly soluble	None to low (breakdown of proteins and nucleic acids directly produces ammonia)
Urea	Mammals Most adult amphibians Marine bony fish	Moderate water solubility Terrestrial environments with a low water content and marine environments can be inhabited.	Very low (100 000 times less toxic than ammonia) Can be stored in high concentrations safely Much less water is lost through urea excretion than through diluted ammonia excretion.	High (ammonia is converted to urea in the liver)
Uric acid	Birds Terrestrial reptiles	Does not dissolve in water (insoluble) Terrestrial (little water is required for the excretion of uric acid as a semi-solid paste)	Relatively non-toxic	Highest (more ATP is required than in converting ammonia to urea)

Key concept

Excretion removes nitrogenous waste products from animals in different concentrations, depending on where the animals live. Aquatic animals continuously excrete dilute urine containing ammonia. In animals that need to conserve water, ammonia is converted to a less toxic form (urea or uric acid) for storage, and the nitrogenous waste is more concentrated to reduce water loss.

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Question set 11.2

REMEMBERING

1 State the type of food and the substances that are broken down into nitrogenous wastes.
2 What are the three types of nitrogenous waste excreted by organisms? Explain why it is essential to remove this waste.

UNDERSTANDING

3 Compare the energy costs of excreting the three different types of nitrogenous wastes.

APPLYING

4 Explain why different groups of animals have different nitrogenous waste products.

11.3 KIDNEYS MAINTAIN WATER BALANCE

Maintenance of water balance in mammals is controlled by **antidiuretic hormone (ADH)**. As the name suggests ('diuresis' means excessive urination), ADH reduces urine output. ADH is produced by the hypothalamus and is stored in the pituitary gland. It reduces urine output by acting on the collecting ducts of the kidney. The main functions of the collecting ducts are reabsorption of water and carrying urine to the ureter. ADH increases reabsorption of water in the collecting ducts.

When the blood water content falls below its optimal range, osmoreceptors in the hypothalamus detect an increase in blood solutes (created by the low water content in the blood) and it causes ADH to be released by the posterior lobe of the pituitary gland. The ADH is secreted into the blood for transport to the kidneys. ADH increases the permeability of the collecting duct of the kidney, increasing water reabsorption. As water concentration increases in the blood plasma, negative feedback decreases the release of ADH.

Osmoregulation is used to keep the bodily fluid from being too diluted or too concentrated. When the bodily fluid is too concentrated, there are high levels of solutes and a low water content. This leads to high osmotic pressure and the risk of cells losing too much water via osmosis. When the bodily fluid is too dilute, there are relatively low levels of solutes and high water content. When this level exceeds the tolerance range, cells are at risk of absorbing too much water, which can result in them lysing (bursting) in the case of animal cells.

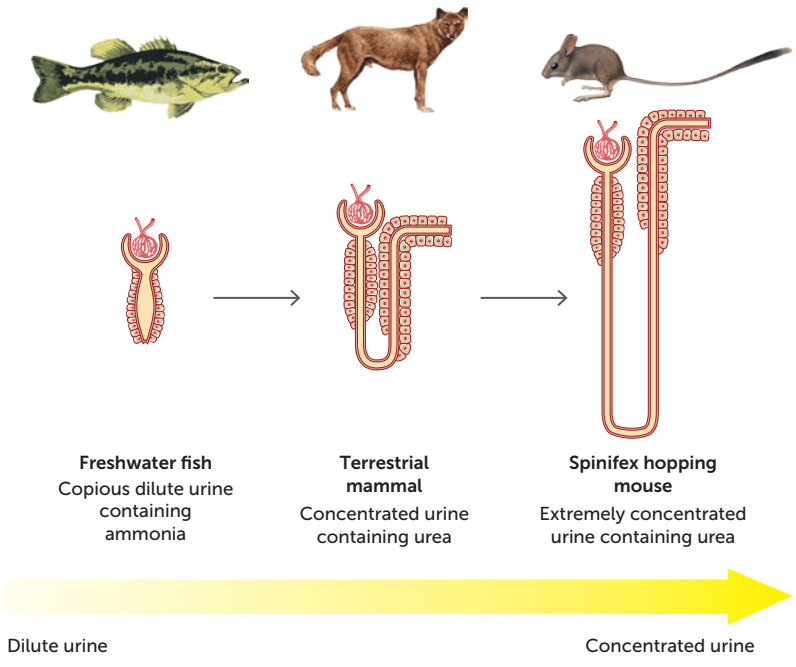

FIGURE 11.3 Urine concentration varies between animals, depending on their environment.

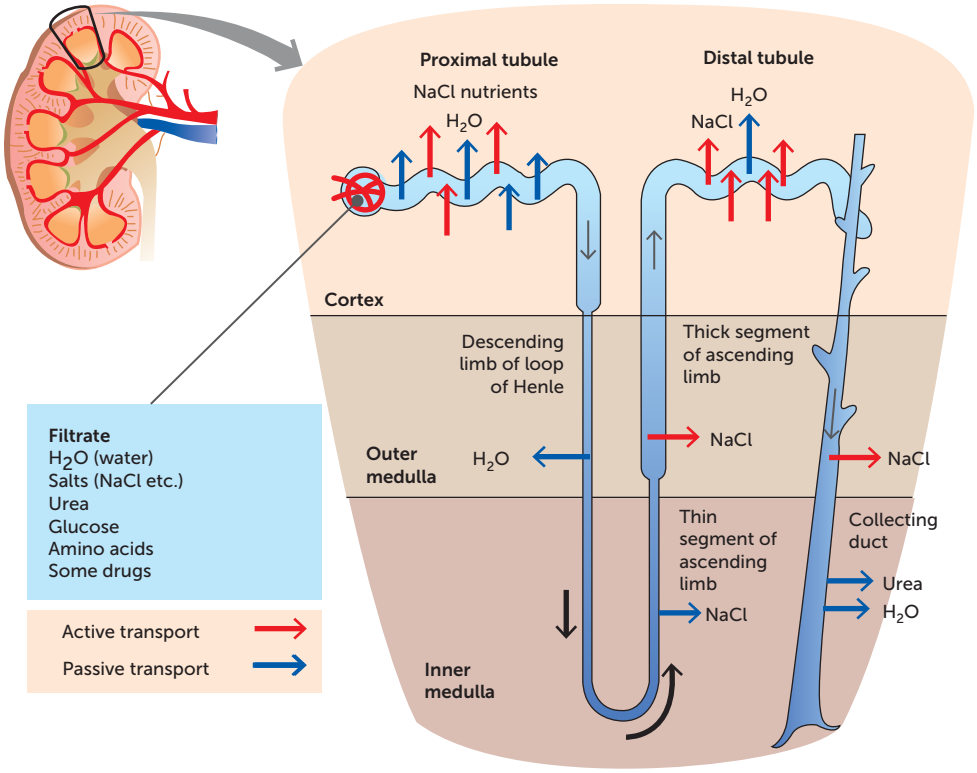

FIGURE 11.4 Water is conserved in the mammalian kidney by reabsorption in the descending portion of the loop of Henle. The longer the loop of Henle, the more water is reabsorbed and the more concentrated the urine.

Negative feedback loop for water balance

As a response to an increase in solute concentration in the blood (less water in the blood), the hypothalamus signals the urge to drink and also sends a nerve signal to the pituitary gland, instructing it to release stored ADH into the blood, which mainly targets cells in the collecting ducts in the kidney nephrons. This **hormone** increases the permeability of the collecting duct walls (opening the water channels) to allow water to flow back into the bloodstream (reabsorption). This increases the water content of the blood and reduces water loss through urine. The resulting low volume of urine is highly concentrated.

The reverse can occur when the water content of the blood is above the tolerance range. The hypothalamus detects this deviation from normal (the stimulus) and coordinates a response. It sends a message to the pituitary gland to release less ADH, reducing the permeability of the collecting duct, which in turn reduces the amount of water reabsorbed back into the blood. The resulting large volume of urine is highly dilute.

When water levels in the blood are returning to the optimal range, the amount of ADH released and the urge to drink are reduced by negative feedback.

Key concept

The hypothalamus detects low water content in the blood and signals the pituitary gland to release ADH. Increased ADH increases water reabsorption by the kidneys, which decreases water loss by urination.

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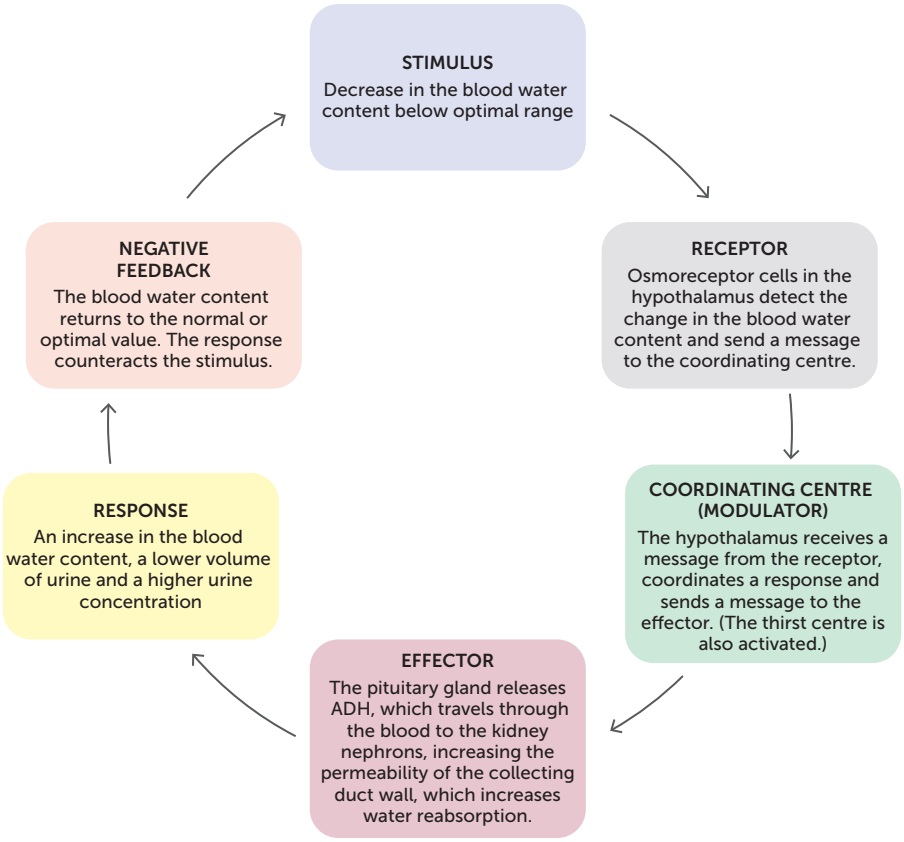


FIGURE 11.5 Negative feedback loop for low water volume (low hydration)

Negative feedback for water content
Read and use the interactive tool to learn about ADH and control of water balance.

Question set 11.3

REMEMBERING

1 State the full term represented by the acronym ADH.
2 State the relationship between the length of the loop of Henle in the nephron and the urine concentration that is demonstrated by Figure 11.3 (page 377).

UNDERSTANDING

3 Explain how reabsorption of water can be controlled in a mammal.

APPLYING

4 Draw a negative feedback loop for an increase in the water content of the blood above the optimal range.

11.4 ADAPTATIONS FOR WATER BALANCE

Organisms have various mechanisms for maintaining water balance. Some regulate their solute concentration to be either higher or lower than that of their external environment; these organisms are called **osmoregulators**. Others allow their solute concentration to be equal to the concentration of the external environment; these organisms are called **osmoconformers**.

Osmoregulators

Structural features, as well as behavioural and physiological responses, aid in thermoregulation. In hot environments, dingoes pant, losing water vapour from the tongue, the air passages and the lining of the mouth. Other animals have high densities of sweat pores in certain areas, which are exposed

as the body temperature rises. These are effective cooling adaptations, but also involve water loss by evaporation. Fortunately, a physiological thirst response is experienced as the concentration of blood solutes increases, and animals respond by drinking water. Thermoregulation and osmoregulation are intricately bound to each other, and for many terrestrial organisms, a water supply is not always available. Animals living in dry areas have a range of structural, physiological and behavioural adaptations for maintaining their water balance.

Structural features to maintain water balance

The threat of dehydration is a major issue in Australian terrestrial animals. Structural adaptations that reduce water loss are essential. A waterproof or impermeable outer layer (integument) can reduce water loss. For example, the scales of reptiles, the hair of mammals, the feathers of birds and the upper part of the epidermis contain keratin, a protein that hardens and waterproofs the body surface. The waterproof surface acts as a barrier, preventing water loss via osmosis or evaporation.

FIGURE 11.6 The scales of reptiles and feathers of birds contain keratin, and this reduces water loss.

Physiological processes to maintain water balance

Many reptiles and birds reabsorb water from their cloaca, the cavity into which their rectum and ureter open. Excreting nitrogenous waste as uric acid is effective in saving water. Many terrestrial vertebrates, such as the Australian desert frog, *Chiroleptes*, slow down the production of urine by reducing the rate of glomerular filtration. The frog, swelling up like a ball, retains urine in its bladder for use in the dry season. The Australian desert hopping mouse, *Notomys alexis*, can concentrate its urine more than any other known rodent (see Figure 11.7). Water is conserved when it is reabsorbed in the descending portion of the loop of Henle. The longer the loop of Henle, the more concentrated the urine and the more water saved. The desert hopping mouse has a very long loop of Henle to maximise water conservation, and both the desert hopping mouse and the kangaroo mouse are able to conserve water by producing a low volume of highly concentrated urine.

Some desert mammals do not need to drink water. They extract enough water from the food they consume, such as plants that store water. Some animals can use water stored in fats or carbohydrates, or use the water that is generated from the **metabolism** of fats or carbohydrates.

Camels are renowned for their ability to go for several weeks without drinking water. Camels can produce water by metabolising the fat in their hump; however, that source of water is not enough to compensate for the water lost by evaporation. The camel's body fluids become increasingly concentrated. The camel can survive, however, because its tissues are extremely tolerant of this condition.

FIGURE 11.7 The desert hopping mouse, *Notomys alexis,* conserves water due to its very long loop of Henle.

Marine vertebrates have body fluids that tend to be hypotonic to their surroundings; that is, their body fluids are of a lower concentration compared with the medium in which they live. Water is lost via osmosis from the gill surfaces. Therefore, marine fish drink copious amounts of seawater. The problem this creates is the additional salt intake. Marine vertebrates are able to solve this problem by the active removal of salts by special chloride-secreting cells in their gills. In addition, a slow filtration rate and the excretion of concentrated nitrogenous waste help them reduce water loss. Some marine animals, such as sharks and rays, tolerate high levels of urea in their body tissues, thereby reducing their water loss. In this way, the internal solute concentration of their tissues becomes slightly higher, or hypertonic, compared with the surrounding water. The water that consequently moves in due to osmosis is easily removed by the kidneys.

Freshwater vertebrates have a concentration of ions in their tissues that is higher than that of the surrounding water. They have a high rate of kidney filtration and produce copious amounts of dilute urine. Freshwater fish must actively absorb salts from their external environment in order to maintain their high ion concentration levels. Some fish, such as salmon, migrate between fresh water and seawater. They can change their osmoregulation mechanisms to suit the different environmental problems.

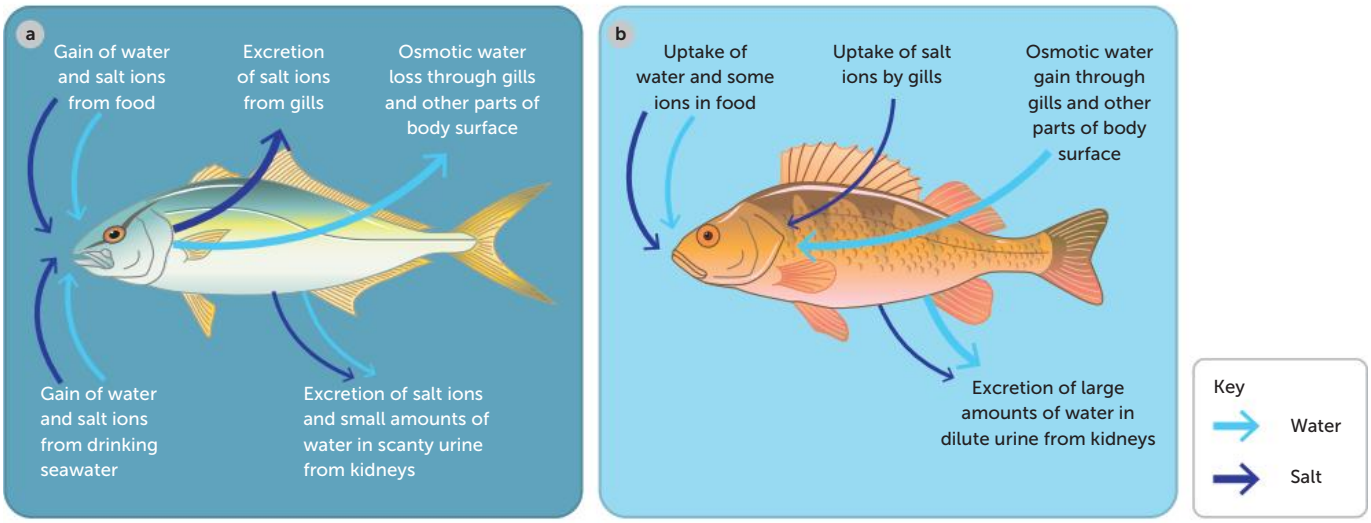


FIGURE 11.8 Solving the problem of water balance in **a** marine bony fish and **b** freshwater bony fish

TABLE 11.2 Problems and adaptations of freshwater and marine bony fish

FACTOR	MARINE BONY FISH	FRESHWATER BONY FISH
Problems	Loses too much water via osmosis across the skin. Gains too many salts by drinking seawater and eating food.	Gains too much water via osmosis across the skin and when eating food containing water. Loses too many salts via diffusion and in urine.
Adaptations for water balance	1. Constantly drinks seawater 2. Eats food containing water 3. High level of reabsorption in kidneys 4. Excretes a low volume of highly concentrated urine	1. Does not drink water. (Fish swim with their mouth open so that water passes by their gills for gas exchange, but they do not swallow.) 2. Low level of reabsorption in kidneys 3. Excretes high volumes of dilute urine
Adaptations for salt balance	1. Excretes highly concentrated urine, ridding the body of excess salts 2. Active transport of salts from salt-secreting cells in gills to the seawater	1. Gains salts when eating food 2. Active uptake of salts from seawater across gills

Behaviours for retaining water: aestivation and burrowing

Desert frogs have adaptations ranging from the production of highly concentrated urine to burrowing in the desert sands for several months at a time. For example, the water-holding frog, *Litoria platycephala*, fills its bladder and pockets under the skin with water and tucks itself into a water-conserving cocoon created from mucus and sloughed skin. The frog's metabolic rate slows, enabling it to enter aestivation in this cocoon under the ground. Water is a product of some metabolic processes, so slowing down the metabolic rate can reduce water loss. The frog can survive in this way for many months.

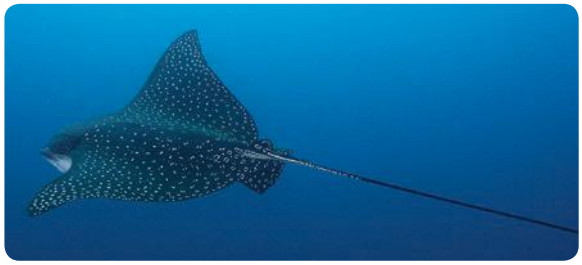
Other desert animals spend a large proportion of time in burrows. Burrows have lower temperatures and higher humidity than the open air, so water loss is reduced. The burrow also traps exhaled water vapour, so there is a lower concentration gradient of water vapour between the air and the animal when it is in the burrow, which leads to less evaporation and less water loss. The desert hopping mouse, *Notomys alexis*, has a bushy end to its tail, which it wraps around its face, trapping the moisture from the air it breathes out. This interesting behavioural adaptation reduces water loss by saturating the air between its face and the bushy tail.

Auscape. All rights reserved/Kathie Atkinson

FIGURE 11.9 A water-holding frog, *Litoria platycephala*, breaking from its cocoon after rain

Osmoconformers

Most marine invertebrates, such as cnidarians and molluscs, are osmoconformers. Their interstitial fluid concentration fluctuates to match that of the external environment. An organism whose body fluids are of the same concentration as the surrounding water is referred to as isotonic. Cartilaginous fish



Nature Picture Library/Brandon Cole

FIGURE 11.10 Rays are osmoconformers.

such as sharks and rays are also osmoconformers. They are able to concentrate urea in their bodies to maintain a high solute concentration, thus matching the ocean's high concentration of solutes. Some fish, such as sturgeon, have the capacity to conform to salt water and fresh water and can live in water with variable salinity, such as in **estuaries**.

Key concept

Animals can be described as osmoregulators or osmoconformers. Osmoregulators *regulate* their internal osmotic concentration to be less than or higher than their environment. Osmoconformers *conform* to the osmotic concentration of their environment; that is, their internal osmotic concentration matches that of their environment. Osmoconformers are isotonic.

Osmoregulation and communication

Animals are multicellular organisms that use a variety of chemical and electrical signals within a communication network, coordinating the way individual cells support the organism as a whole. The communication network involves two systems: the nervous system and the endocrine system. Rather than being two separate systems, they are integrally related and follow the same principles of cell-to-cell communication. In both systems, signalling molecules are produced and released by particular cells, to signal to other cells. Other cells respond to these signalling molecules and effect a change in cell functioning.

The endocrine and nervous systems are inextricably linked through the hypothalamus and pituitary gland. The hypothalamus, which is made up of nervous tissue, is located in the brain and is connected to the pituitary gland via both nerves and blood. An example of the interplay between the endocrine and the nervous systems is seen in the regulation of the concentration of water in the blood plasma. Osmoreceptors, sensory neurons that detect the water content of the blood, are located in the hypothalamus. If the water content of the blood is low, the osmoreceptors send a nerve signal to the pituitary gland to release stored ADH into the bloodstream, through which it travels to the kidneys. In the kidneys, ADH makes a change in cell behaviour, increasing the reabsorption of water.

Salt balance

Salts are needed for the proper functioning of muscles, nerves and bones. The salts are usually dissolved in water. Animals need to regulate the salt lost in sweating, which is an essential process for cooling the body. Salts can be replaced through the diet, and they can also be reabsorbed via the nephrons. Excess salts need to be excreted via the excretory system. Animals such as the albatross have evolved a way to drink seawater, which is too salty for most birds and land animals. To get rid of excess salt from the water and food they ingest, albatrosses have salt glands just behind their eye sockets. The glands excrete a highly concentrated salt solution that drains out through the tip of the beak.

Question set 11.4

REMEMBERING

1. Describe the difference between an osmoconformer and an osmoregulator.
2. Create a table summarising the adaptations osmoregulators use to maintain water balance.

UNDERSTANDING

3. Contrast the problems marine and freshwater fish have in their different environments in terms of water and salt balance.
4. What is one benefit of being an osmoconformer rather than an osmoregulator?
5. Explain why excretion is important in order to achieve water and salt (osmotic) balance.

SCIENTIFIC LITERACY

Water balance in different organisms

All organisms need to regulate water and salts. Different organisms exhibit different types of osmoregulation, but they all involve a stimulus and a response.

Fish respond based on whether they live in a freshwater or marine environment. Freshwater fish are hypertonic to their surroundings. The concentration of salts is higher in their blood compared with that in the surrounding water. Controlling intake of food and water, excreting high volumes of dilute urine, and actively absorbing salts into cells work together to regulate water and salt concentration in freshwater fish. Marine fish are hypotonic to their surroundings. The concentration of salts is lower in their blood compared with in the surrounding water. A combination of controlled intake of food and water, excretion of low volumes of concentrated urine and active secretion of salts from cells contributes to water and salt regulation in marine fish.

Bacteria are tiny unicellular organisms. They use an active transport mechanism to absorb salts when they are hypotonic to their surroundings.

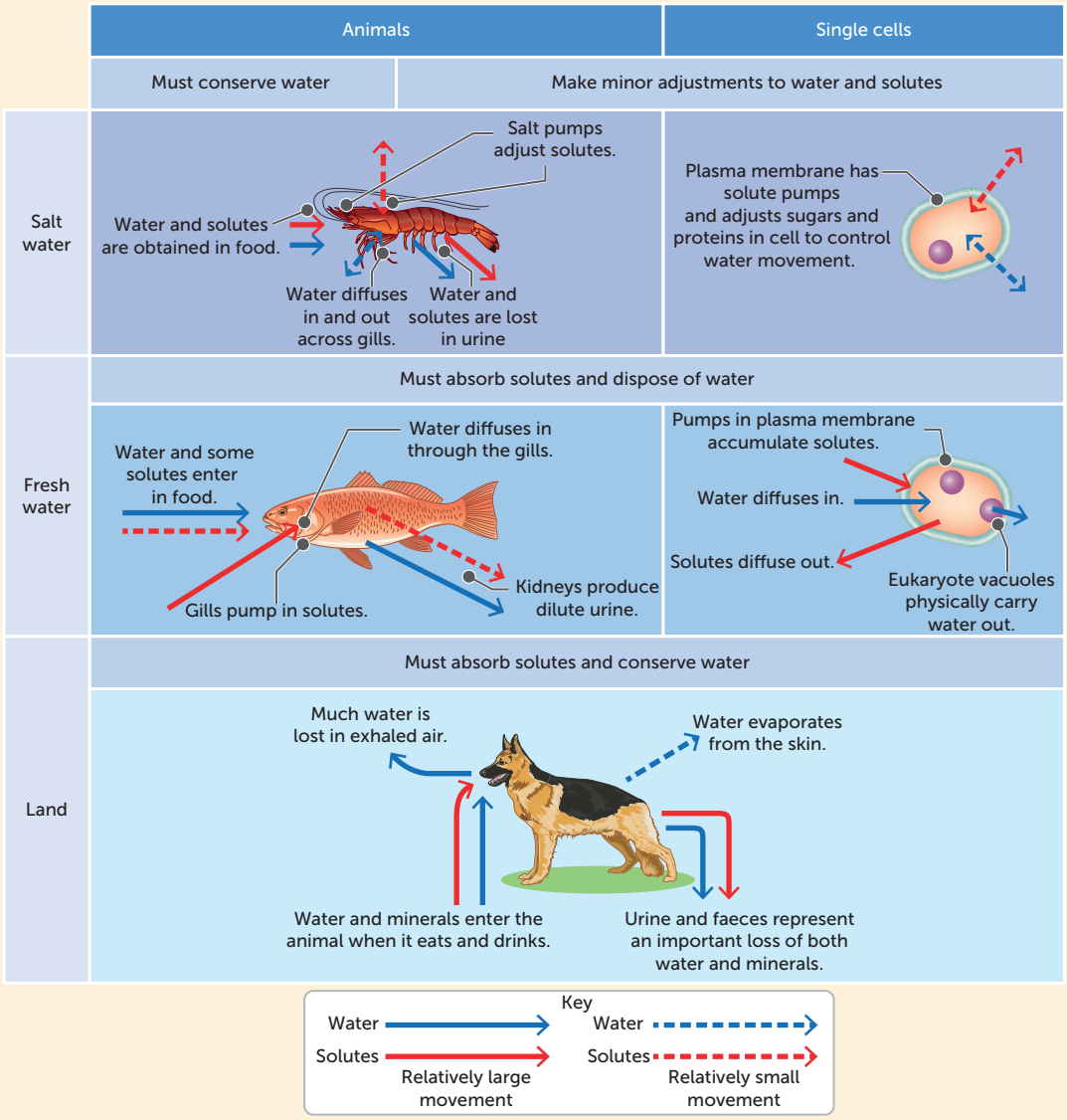


FIGURE 11.11 Water is gained and lost differently by organisms in water and on land.

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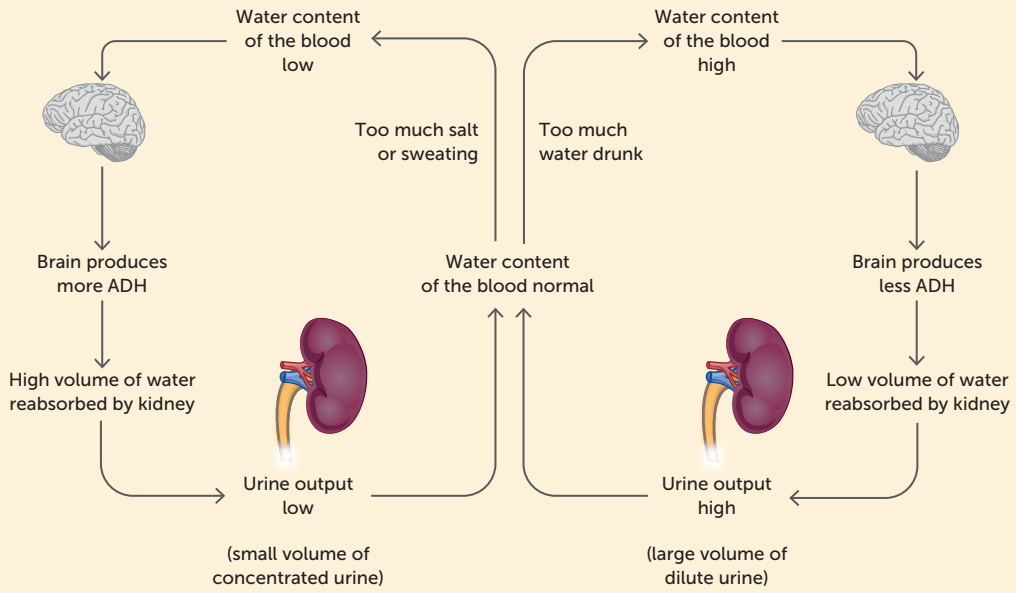


FIGURE 11.12 Negative feedback loops for high and low water content in blood

Plants have very specialised mechanisms for water and salt regulation. For example, they use stomata (singular stoma) and their accompanying guard cells (on the lower side of their leaves) to regulate water loss. The stomata can open and close in response to the concentration of the solutes in their guard cells.

Most mammals have a highly developed excretory system to help regulate water and salts. They use their nervous and hormonal communication systems to regulate urine output volume and concentration. Some mammals, like koalas, rarely drink water. Instead they can extract water from the food they eat (eucalyptus leaves). During very dry times, droughts and bushfires, they will be seen drinking water.

Questions

1. List the types of animals that need to conserve water.
2. List the types of animals that need to dispose of water.
3. Compare the mechanisms for water and salt regulation of a fish with those of a human.
4. Write a sequential summary of the negative feedback loops that operate when a human's water content is too high or too low.

11.5 WATER TRANSPORT IN PLANTS

An understanding of basic plant structure and function is required in order to understand how a plant regulates water and salt. You may recall that roots, stems and leaves are areas that all require water and salts to be within a tolerance range. Water is gained through the root hairs and lost mainly through the stomata in the leaves.

Transport and transpiration lead to water loss

Roots have fine root hairs attached to them that have an extremely high surface-area-to-volume ratio. They can achieve high rates of osmosis and diffusion, as well as active transport of various substances. Water and dissolved substances, such as salts, are transported up the stem. Inside the

stem of a **vascular plant** are the transport tissues, **xylem** and **phloem**. The xylem carries water and the phloem carries the products of photosynthesis, such as glucose. The stem is attached to leaves. The leaves consist of layers of specialised tissue and are the site where the majority of water loss occurs in a plant (through **transpiration**).

Water transport and water loss happen simultaneously in vascular plants. Water is pulled from the roots through the xylem to the leaves due to the set of forces known as the **transpiration pull**. These forces include the forces of **cohesion** and **adhesion**. Cohesion is the attractive force that occurs between water molecules. As water evaporates from the leaves, columns of water are drawn up through the xylem vessels. Adhesion is the attractive force operating between water molecules and the inner walls of the xylem vessels.

The combined forces of cohesion and adhesion create **capillary action**. Capillary action is defined as the movement of water within the spaces of a porous material due to the forces of adhesion and cohesion. As water continues to move up the column and is drawn from the root hairs (by the xylem and the water molecules it contains), this sets up a concentration gradient between the inside and outside of the root hairs, enabling water to move in by osmosis. In addition, active transport of salt ions into the roots can cause osmotic water movement into the root hairs, balancing the salt concentration inside and outside the root hairs. This movement of water into the root hairs causes **root pressure**, a force that pushes the water upwards.

Together, the forces of cohesion, adhesion and root pressure produce a continuous flow of water from the roots to the leaves via the xylem. This continuous flow of water is known as the **transpiration stream**.

The strongest force, the cohesive force, causes a pull force in the xylem, because water at the top of the column, at the leaf, evaporates due to a process called transpiration (Figure 11.13). Transpiration is the evaporative loss of water (in the form of water vapour) from plants, usually through small pores called stomata found on the surface of a plant, mostly on the underside of leaves. The evaporation and diffusion out of the stomata occurs because of the concentration gradient of water vapour between the inside and outside of the leaf. Water vapour moves down the concentration gradient, from an area of high water content to an area of relatively low water content. The transport of water through the plant results in water loss.

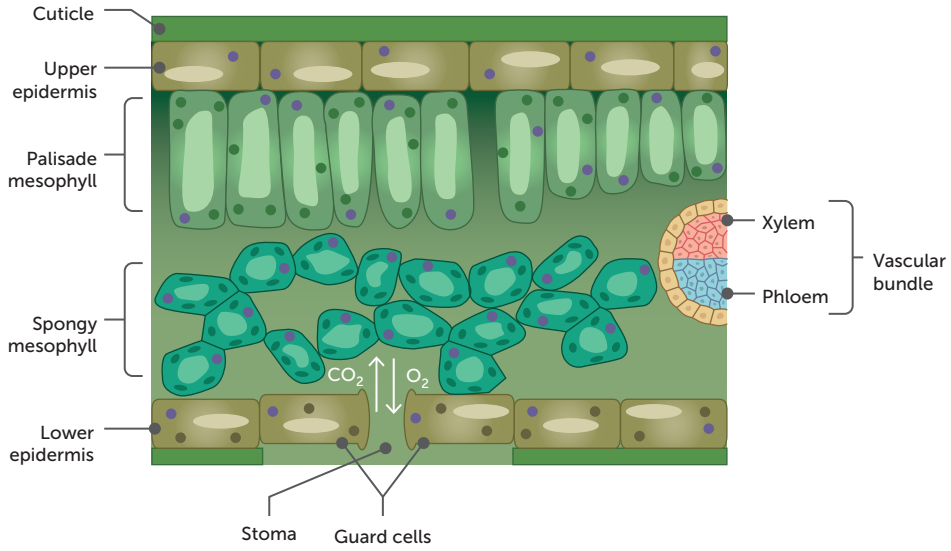
Source: USGS USA.gov

FIGURE 11.13 Evidence of transpiration is shown in the results of this experiment. A plastic bag was wrapped around some leaves on a plant. Water vapour from transpiring leaves can be seen on the plastic bag.

The importance of transpiration

1. Transpiration supplies photosynthesis with the water it needs.
2. The evaporation of water from the mesophyll cells (Figure 11.14) in the leaves that accompanies transpiration requires energy and therefore cools the leaves in the same way that sweating cools the skin of some mammals. Heat energy is drawn out of the plant, into the water, then out into the external environment.
3. The transpiration stream is also necessary for distributing mineral salts throughout the plant.

Stomata have guard cells that open or close the stomata, depending on environmental conditions, giving the plant some control over water loss. In most plants, light (in combination with other factors, such as sufficient carbon dioxide concentration and sufficient humidity) stimulates opening of the stomata. Using active transport, potassium ions (K^+) are purposely moved into guard cells. This creates a concentration gradient. The guard cells then take up water by osmosis and become turgid. Because their inner walls are rigid, they are pulled apart, opening the pore. In darkness, water is lost, the guard cells become flaccid, and their inner walls move together, closing the pore.



Transpiration quiz
Try this transpiration quiz to find out whether you have learned enough facts.

FIGURE 11.14 Leaf cross-section. The stomata release oxygen and take up carbon dioxide but also lose water.

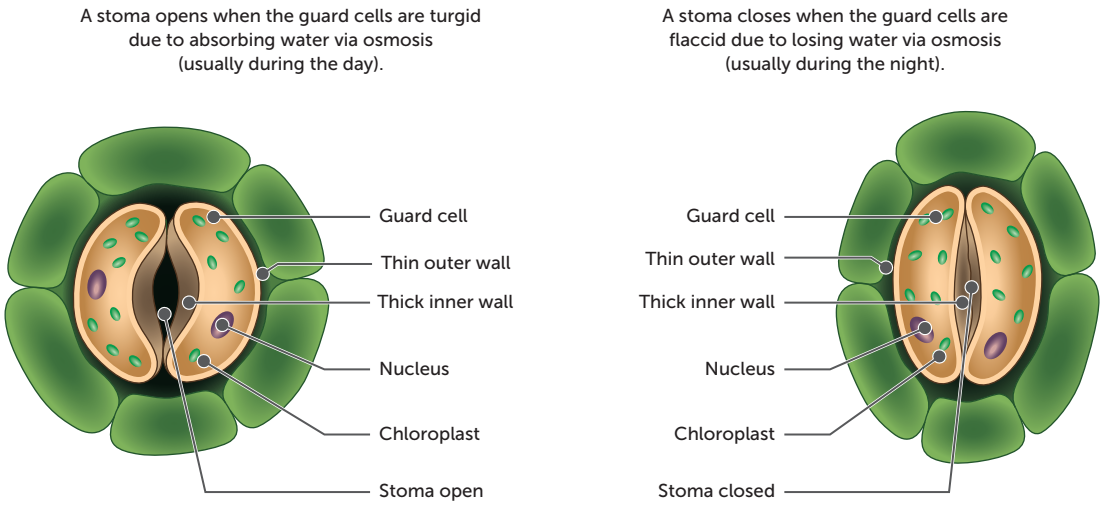


FIGURE 11.15 Comparison of an open stoma and a closed stoma

TABLE 11.3 Factors that can increase the rate of transpiration in plants

Light	An increase in sunlight leads to an increase in transpiration due to warming the leaf and stimulating the opening of the stomata (active transport of ions into the guard cells can cause water to be absorbed via osmosis because of a concentration gradient in the ions in solution); once the stomata are open, transpiration can start.
Humidity	A decrease in humidity leads to a higher water vapour concentration gradient between the air at the surface of the leaf and the air outside the leaf. This increases diffusion of water vapour out of the leaf and evaporation from the leaf surface, which leads to an increase in water loss from the plant (transpiration).
Wind	An increase in wind leads to an increase in the rate of evaporation, which leads to an increase in the rate of transpiration, because humid air near the stomata is being carried away, increasing the water vapour concentration gradient between the air at the surface of the leaf and the air outside the leaf.
Temperature	An increase in temperature (due to heat energy being received from the sun) increases the evaporation rate from the surface of the leaf, because of an increase in the water vapour concentration gradient between the air at the surface of the leaf and the air outside the leaf. This leads to an increased rate of water loss from the plant (transpiration rate).

Key concept

The mechanisms of osmoregulation in plants are transport and transpiration. Transport describes the movement of water and minerals in the xylem up the stem to the leaves, and solutes in the phloem. Transpiration stream describes the pull of water from the roots to the leaves due to cohesion, adhesion and root pressure. Transpiration is the loss of water from the leaves.

CASE STUDY

While a PhD research student at the University of Western Australia in 2016, Louis Moir-Barnetson conducted a study on different halophyte (salt-tolerant) species surviving in the salt lakes of inland Australia. The environment is described as highly stressful due to the high salinity, extremely arid conditions and flash floods. The study was conducted because little is known about the ecology and physiology of the salt lake plants. Their adaptations are key to their survival in this extreme environment, and their survival is vital for the ecology of the salt lake. Halophytes seem to have abilities

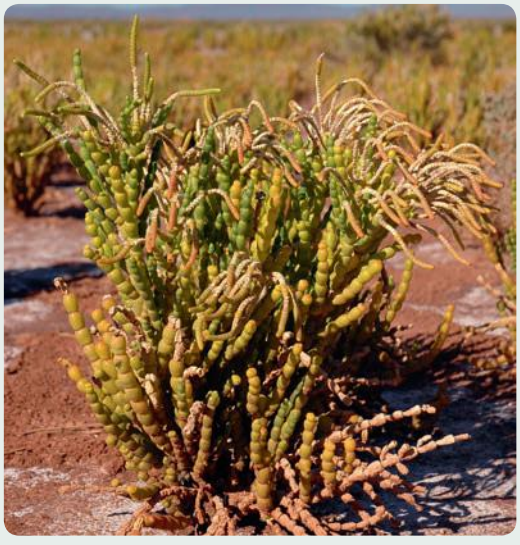


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FIGURE 11.16 Highly specialised plants can survive in extreme conditions; for example, the halophyte *Tecticornia medusa* (samphire) is able to tolerate the highly saline, extremely arid conditions of inland salt lakes.

that other plants (and animals) do not. The ability to filter salt from salt water is of particular interest to scientists, because it is predicted that fresh water will become less common in the future.

Louis also conducted a comparison study of three different halophytes and found that in altered, increasingly saline conditions, shoot and root growth remained the same, but that when subjected to low saline conditions, one suffered more stress than the other two. Samphire was one of two species that he described as superior competitors.

Common mechanisms of halophytes for surviving in environments where there are high concentrations of salt are accumulation and storage of salt in either vacuoles or succulent tissues. When salt is moved into a vacuole, its toxic effects are removed from the rest of the cytoplasm. Importantly, the limiting effects of salt in the cytoplasm disappear and nutrient uptake increases. Samphires accumulate salt in the older parts of the stem, which then fall off, removing the salt from the plant.

Questions

1. Explain why studies of halophytes may benefit humans in the future.
2. Predict some of the impacts that removing halophytes from the salt lakes of inland Australia would have on the ecology there.
3. Describe the halophyte samphire and state some of its unique adaptations.

Question set 11.5

REMEMBERING

1. State the relationship between the following factors and the transpiration rate:
   - a high light intensity
   - b low temperature
   - c high wind
   - d low humidity.
2. Describe the role of the guard cells in regulating water loss in a plant.

UNDERSTANDING

3. Differentiate between the terms 'transpiration' and 'transpiration pull'.
4. Explain why transpiration cannot continue without evaporation.

CREATING

5. Create a poster showing a cross-section of a leaf and the position of stomata on the leaves. Include plant parts and processes you learned about in this section.

11.6 SPECIALIST PLANT ADAPTATIONS FOR REGULATION OF WATER, SALTS AND GASES

To maintain water balance and allow for gas exchange, **xerophytes** (plants tolerant of an **arid** environment) and **halophytes** (plants tolerant of salt) have a variety of adaptations. Adaptations fall into the categories of structural (physical) or physiological (functional processes). Another way to classify plant adaptations relates to the part of the plant involved: e.g. leaf, stomata or root adaptations. When learning about adaptations, it is helpful to be able to describe how the adaptation assists in reducing water loss or salt loss or gain, while maintaining adequate gas exchange.

Gas exchange occurs through stomata, but only when they are open. They are usually open during the day, when sunlight is being used in the process of photosynthesis. Stomatal opening and closing depends on changes in the **turgor** of the guard cells. Turgor is a force that results from the water pressure inside plant cells and it is maintained by osmosis. When water flows into

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the guard cells by osmosis, their turgor increases and they expand. Due to the relatively inelastic inner walls of guard cells, they bend and draw away from each other, so the pore opens. If the guard cells lose water, the opposite happens and the pore closes. The guard cells lower their water potential to draw in water from the surrounding epidermal cells by actively accumulating potassium (K^+) ions. This requires energy in the form of ATP, which is supplied by the chloroplasts in the guard cells.

Plants require oxygen for respiration and carbon dioxide for photosynthesis. Respiration occurs throughout the day and night, providing the plant with a supply of energy. Photosynthesis can only occur during sunlight hours, so it stops at night. During the day, photosynthesis can occur 10 or even 20 times faster than respiration (depending on the light intensity), and the stomata must stay open so that the plant has enough carbon dioxide, most of which diffuses in from the atmosphere. Simultaneously, oxygen, a product of photosynthesis, diffuses out through the stomata. The rate at which oxygen is produced in photosynthesis is much higher than the rate it is needed in respiration. This explains why oxygen is released, even though oxygen is needed for respiration. How can plants that live in dry environments regulate water balance when they need to open their stomata for gas exchange (which also allows evaporative loss of water via transpiration)? Xerophytes have developed specialised features to solve this dilemma.

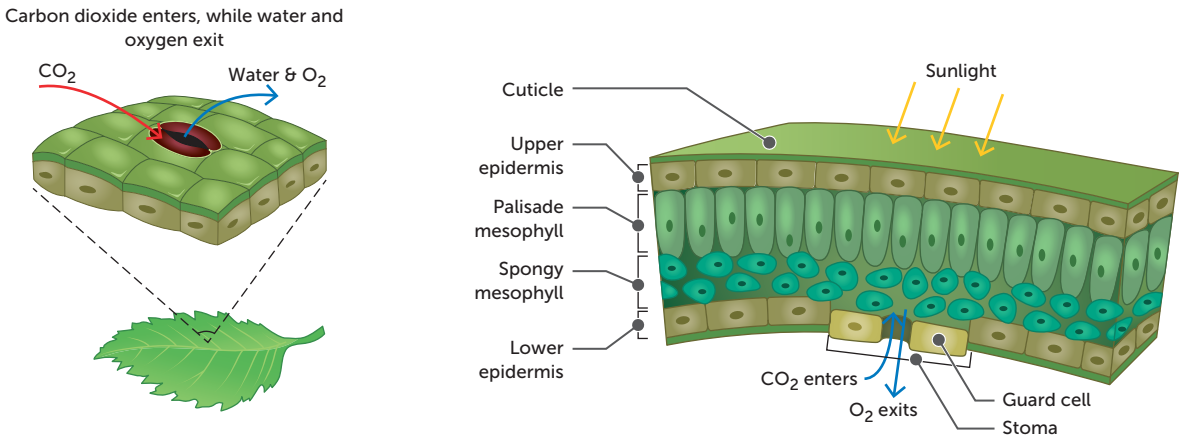

FIGURE 11.17 Gas exchange in plants occurs through the stomata.

Xerophytes

Xerophytes live at the dry extreme of the moisture continuum. Deserts, but also aerial rainforest niches and frozen arctic tundra, experience conditions in which evaporation exceeds precipitation for all or part of the growing season. Xerophytes specialise in water conservation, allowing them to thrive in these conditions. Xerophytes are plants adapted to live in arid environments. They have developed specialised features that minimise water loss, while allowing for gas exchange. An environment is classified as arid if it has a severe lack of available water that hinders the growth of most plant and animal life. 'Xero' is the Greek word for dry; hence the term xerophyte. Xerophytes may live in very hot places, such as the desert, where water is limited, or in areas of frozen land with no flowing water.

The problem with living in an arid environment is that water moves passively along a concentration gradient out of the plant and into the dry environment. The rate of water loss by evaporation due to transpiration is very high. It is high because there is a relatively high concentration gradient between the inside and outside of the leaf. Water vapour evaporates and diffuses more

quickly in an arid environment compared with in a non-arid environment. Plant cells can become flaccid, and plants can wilt, dry out and die when their water content falls below the plant's tolerance range. Water is a requirement for photosynthesis, it is a medium for metabolic processes (chemical reactions), it is required for evaporative cooling, and it is needed for soil nutrients to dissolve into and be absorbed by a plant.

Xerophytes have a range of structural and physiological adaptations that enable them to survive in an arid environment:

- *Reduction in leaf surface area*. Leaves may be reduced to spines, or be long and narrow, reducing the area for transpiration, and there may be a reduced number of stomata. In addition, the smaller leaf surface area means less exposure to the drying effects of the wind, reducing evaporation and reducing water loss. For example, some cacti have developed their leaves into thin spines without stomata to inhibit water loss, while the porcupine (spinifex) grass has rolled leaves that create pockets of very humid air
- *Sunken stomata*, which prevent water loss by increasing the relative humidity near each stoma, decreasing the concentration gradient and reducing evaporation and diffusion. This creates a micro-climate
- *Deep roots* to reach water sources underground, such as the water table, which increase water uptake, preventing dehydration of the plant
- *Rolled leaves*, with the stomata inside and the inner surface covered in hairs. The rolled leaf and hairs both serve to trap moist air, thus reducing the concentration gradient of the water vapour, the diffusion rate of water vapour out of the leaf, the evaporation rate and the transpiration rate, creating a humid micro-climate and reducing water loss
- *Thick, waxy leaf cuticle* that is impermeable to water, preventing evaporation and water loss. The cuticle is also shiny and can reflect light, reducing the amount of light absorbed that could transform into heat and increase the transpiration rate. The reduction in light absorption leads to a reduction in the stimuli that open the stomata, further reducing water loss
- *Stomata opening at night* (*reverse stomatal rhythm*). This assists in reducing water loss, because the stomata are closed during the hottest part of the day, reducing transpiration and evaporation. Carbon dioxide uptake is then at night, and it is stored for use in photosynthesis, which occurs in the daytime
- *Shallow, spreading roots* to collect the occasional rainfall, and to increase water uptake and reduce the risk of dehydration of the plant.
- *Storage of water in succulent tissues*. Plants store water in fleshy stems or leaves (instead of it being transpired out of the plant) for use during dry periods. This reduces water loss during hot, dry periods.

These structural and physiological adaptations can be further classified into the following categories: adaptations to increase water gain (spreading shallow roots or long tap-roots that reach the water table), adaptations that limit water loss (leaf, stomatal, metabolic adaptation) and adaptations for water storage.

Halophytes

Halophytes are plants that live in environments of high soil salinity, that is, soil with a high salt concentration – places such as salt marshes and the mud flats of estuaries. The amount of salt-affected land is increasing around Australia, and scientists study salt-tolerant plants to help in agriculture, bioremediation and conservation.

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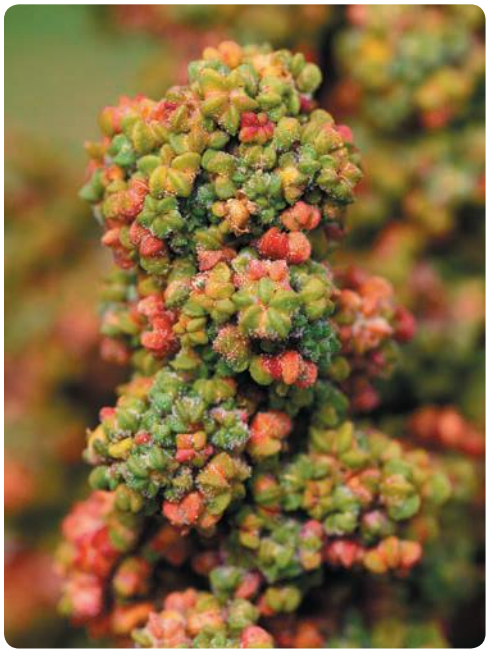
Water does not move into a plant if the plant's solute concentration is lower than the concentration outside. Water will move out of the plant, via osmosis, passively along a concentration gradient. Halophytes lose water, because the high salt concentration in the surrounding soils will draw water from plant tissue via osmosis. The effects of living in an environment with high salinity are multilayered. Plant growth can be reduced, germination can be hindered, and plants can struggle with a water deficit as water is drawn out of them, which slows the rate of photosynthesis and productivity; high levels of salt ions can also lead to toxicity and cell death. Because the salt concentration in the soil exceeds that in the root hairs, unless the plant is specially adapted the water moves from the root hairs into the soil by osmosis until the two solutions are isotonic. Plants can become dehydrated if they do not possess suitable adaptations.

In order to combat the effects of osmosis and reduce water loss, halophytes are salt accumulators and/or salt excluders. Salt accumulators gather and store excess salt in their salt glands or in their central vacuoles. Salt excluders remove salt by ultrafiltration through cell membranes and the endodermis. These complex plants are able to accumulate and compartmentalise ions, enabling them to continue to accumulate essential nutrients in the presence of high sodium concentrations, and limiting the entry of salt ions into the transpiration stream, and hence protecting their ability to carry out transpiration. Some examples of halophyte adaptations follow.

- *Filtration at the roots* to regulate the amount of salt entering the plant. Root cells that are impermeable to salt prevent salt from entering the plant.



Alamy Stock Photo/Sarah Marchant

FIGURE 11.18 Salt glands on a quinoa flower

Science Photo Library/Bob Gibbons

FIGURE 11.19 The desert holly saltbush accumulates salt in its leaves, which can be dropped in order for the plant to survive.

- *Vacuoles in root cells* store salt, which increases the salt concentration of the roots so it is greater than that in the soil. This allows water movement into the roots. Storing salt in vacuoles, rather than in the cytoplasm, stops it from interfering with cell functioning.
- *Accumulation of salt* in older leaves, **salt bladders** (modified epidermal hairs) or bark, which can later be discarded. This reduces the amount of salt in the plant.
- *Secretion of salt by special salt glands* on leaves, stems and roots. This also reduces the amount of salt in the plant.
- *Succulence*, the development of water storage structures in the leaves and other parts of the plant, dilutes the salt content of the cells (as well as giving the plant a water source in drier periods).

Examples of halophytes are mangrove grass and mangrove trees (Figure 11.20). Mangroves are characterised by their aerial root systems, called pneumatophores, which aid in respiration. The muddy, oxygen-poor soils that characterise mangrove areas do not hold enough oxygen for these trees to effectively respire. Pneumatophores help the mangrove plant gain enough oxygen for respiration. They are most common in the Kimberley and Pilbara regions, Exmouth, and Shark Bay. There are also some small and isolated communities at the Abrolhos Islands in the state's mid-west and in the Leschenault Inlet, Bunbury, in the south-west. The diversity of mangroves diminishes markedly from north to south, and only one species is found at Shark Bay and further south.

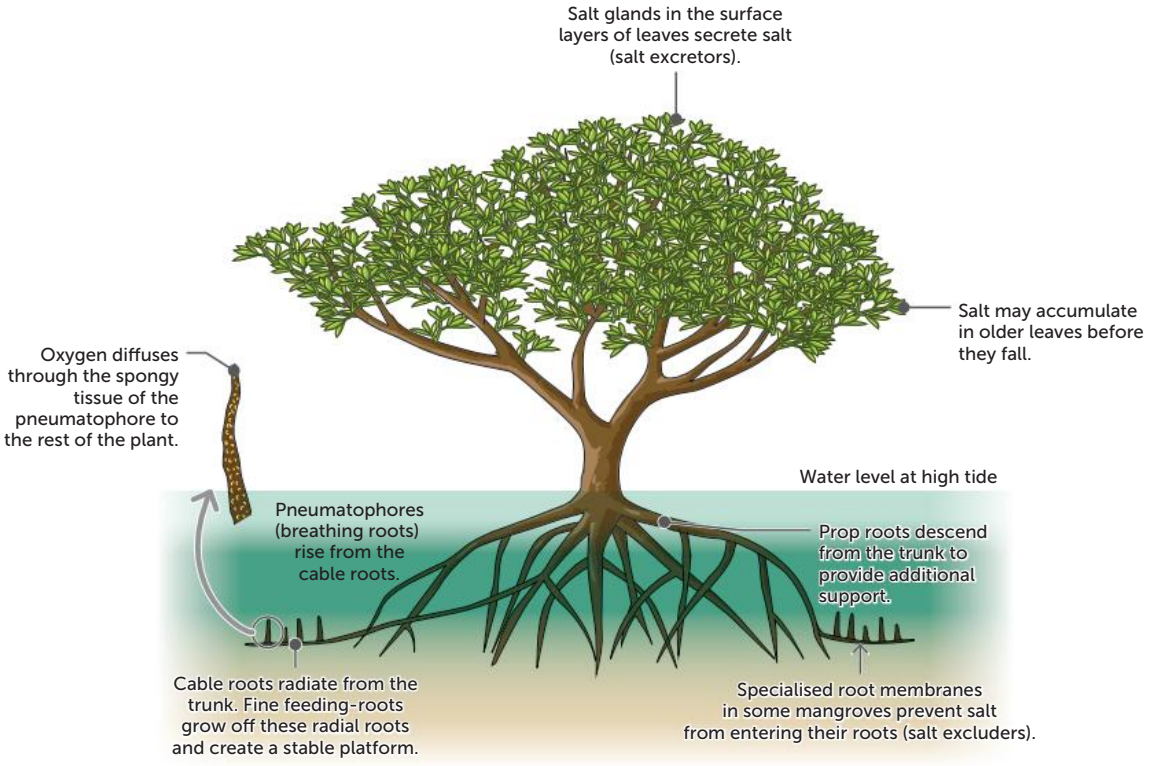


FIGURE 11.20 Mangroves have evolved methods of dealing with concentrations of salt that would kill or inhibit the growth of most other plants.

TABLE 11.4 Structural and physiological adaptations of xerophytes and halophytes

XEROPHYTES			
TYPE OF ADAPTATION	**ADAPTATION**	**HOW IT WORKS**	**EXAMPLE**
Structural	Thick, waxy cuticle	Impermeable to water, preventing evaporation and water loss. Stops uncontrolled evaporation through leaf cells.	 Wikimedia/Kevin Thiele CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/deed.en) **FIGURE 11.21** The Australian succulent *Gunniopsis quadrifida* (Sturts pigface)
	Small leaf surface area	Fewer stomata, leading to reduced water loss. Less surface area for evaporation. Smaller surface area of leaf is exposed to the drying effects of the wind, reducing evaporation and reducing water loss.	Conifer needles, cactus spines
	Sunken stomata Rolled leaves with stomata on the inside	Stomata in sunken pits within rolled leaves prevent water loss by increasing the relative humidity in the vicinity of each stoma, decreasing the concentration gradient and reducing evaporation and diffusion. Creates a micro-climate.	 Wikimedia/Kevin Thiele CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/deed.en) **FIGURE 11.22** Porcupine grass, also known as 'spinifex' or hummock grass (*Triodia* sp.), WA
Physiological	Stomata opening at night (reverse stomatal rhythm)	This assists in reducing water loss because the stomata are closed during the hottest part of the day, reducing water loss by transpiration/evaporation (CO_2 uptake occurs at night and it is then stored for use in photosynthesis during the day).	
	Storage of water in succulent tissues	Plants store water in cells in fleshy stems or leaves instead of transpiring it out of plant, for use during dry periods; reduced water loss during hot dry periods.	*Gunniopsis quadrifida* (shown above in Figure 11.21)

XEROPHYTES			
TYPE OF ADAPTATION	**ADAPTATION**	**HOW IT WORKS**	**EXAMPLE**
Structural	Aerial root systems called pneumatophores	Aid in respiration. The muddy, oxygen-poor soils that characterise these areas do not hold enough oxygen for these trees to effectively respire. Oxygen diffuses into the spongy tissue of the pneumatophores. They grow upwards out of the water or mud to reach the air.	 Shutterstock.com/sasimoto **FIGURE 11.23** Mangroves
	Filtration structures in roots	Prevent salt from entering their roots. Mangroves have an ultrafiltration system that can filter approximately 90% of sodium ions from the surrounding salt water. The three layers of the filtration system surrounding the roots trap sodium ions but allow water to pass through as it is pulled into the xylem.	
	Salt glands	Salt is directed to plant surfaces, where salt glands secrete salt to reduce the salt content in the plant	
Physiological	Concentrates and stores salts in vacuoles	Stores salt in the vacuoles of the fleshy stem segments or 'beads', which can have salt concentrations of 30–45%. The salt in the beads becomes highly concentrated, and they shrivel, die then drop off. This allows the rest of the plant to remain healthy.w	 Shutterstock.com/sasimoto **FIGURE 11.24** Samphire: an Australian succulent
	Accumulates salts in leaves or bark	Salt is directed to older leaves or bark, where it accumulates. The leaves or bark eventually die and drop off, removing the salt from the plant.	

Key concept

Plants have structural and physiological adaptations to help them survive in different environments. Xerophytes are plants that can tolerate extremely dry environments. Halophytes are plants that can tolerate high soil salinity.

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Question set 11.6

REMEMBERING

1 Describe the structural adaptations of these plants:
   a xerophyte
   b halophyte.
2 Describe the effects of an arid and highly saline environment on water balance for plants.
3 Copy and label Figure 11.25.

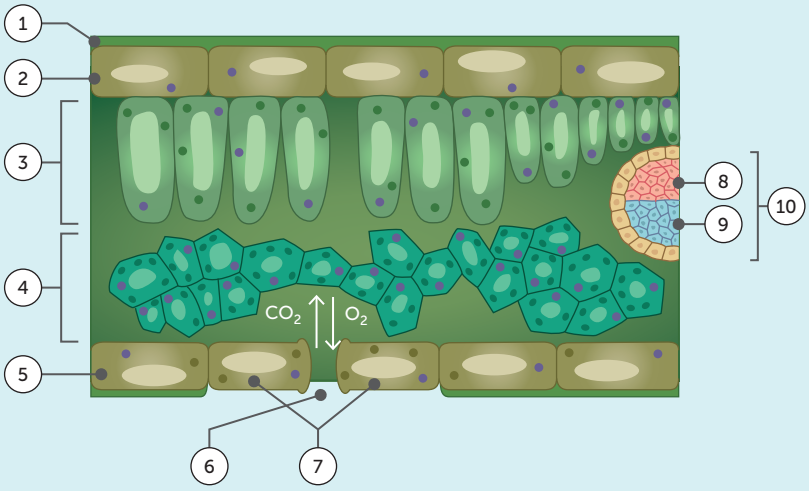

FIGURE 11.25 Cross-section of a leaf

UNDERSTANDING

4 Draw a table to summarise two structural adaptations of xerophytes for reducing water loss and two structural features of halophytes for salt regulation.

CREATING

5 Draw a diagram of a mangrove tree and add notes explaining the various structural and physiological features it has for water, salt, oxygen and carbon dioxide regulation.

11.1 APPLICATION

The problem of osmosis

Simple unicellular organisms, such as *Amoeba*, solve the problem of water gain from osmosis by accumulating the excess water in little bubbles in their cytoplasm. These contractile vacuoles swell to bursting point, and the surplus water is expelled from the cell surface as the vesicular membrane suddenly contracts.

11.2 APPLICATION

Bioengineered kidney makes urine

Scientists can strip cells out of donated organs, such as kidneys, leaving a scaffold. The scaffold can then be populated with stem cells under conditions mimicking those in the body. The end result is a functioning bioengineered kidney that can be transplanted into the stem cell donor.

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CHAPTER 11 INVESTIGATION

11.1 INVESTIGATION

How are animals adapted to withstand cold or heat?

Background

Mammalian body temperatures vary little. What are some of the adaptations that help mammals maintain a fairly constant body temperature and keep warm in cold climates?

Aim

To model and investigate heat loss from an exposed surface

Materials

- 4 test tubes
- 4 thermometers
- 4 beakers
- Funnel
- Measuring cylinders
- Cotton wool (or some other insulating material)
- Cardboard cylinder (such as from a toilet roll)
- Timer
- Fan
- Spray bottle of warm water

Risks

WHAT ARE THE RISKS IN DOING THIS EXPERIMENT?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Hot water can burn.	Use a funnel and fill test tubes carefully.

Procedure – Part A: Effect of insulation on heat loss

1. Take three test tubes, label them 'A', 'B' and 'C', and place each one into a separate beaker.
2. Surround test tube A with cotton wool or some other insulating material.
3. Place test tube B in a cardboard cylinder and wrap the outside of the cylinder with the same amount of insulating material as you used for tube A (so that there is a layer of air between the test tube and the insulation).
4. Cover the top of the cardboard cylinder so the air is trapped.
5. Test tube C has no insulating material around it.

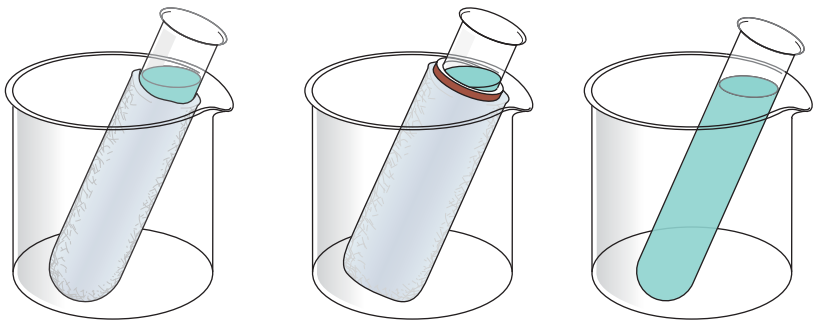

FIGURE 11.26 Experimental set-up to investigate the effect of insulation on heat loss

6 Fill each of the three test tubes with 20 mL water at 80°C.
7 Insert a thermometer in each test tube and record the three temperatures as soon as possible after the water is added. In a table, record the three temperatures every minute for 10 minutes.
8 Graph your results.

Procedure – Part B: Effect of moisture on heat loss

1 Take four test tubes that have been wrapped in cotton wool and place each one in a separate beaker. Label them '1', '2', '3' and '4'.
2 Spray the outside of test tubes 1 and 3 with warm water.
3 Place test tubes 1 and 2 in front of a fan, and test tubes 3 and 4 in an area without air movement.
4 Fill each of the four test tubes with 20 mL water at 80°C.
5 Insert a thermometer in each test tube and record the four temperatures as soon as possible after the water is added. In a table, record the four temperatures every minute for 10 minutes.
6 Graph your results.

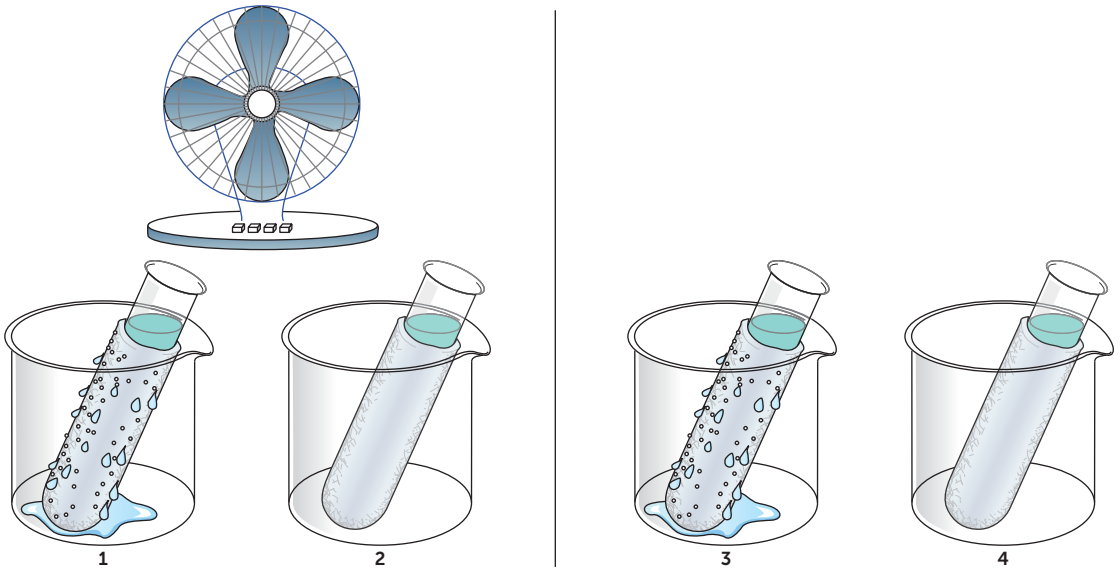

FIGURE 11.27 Effect of moisture on heat loss

Results

Observations are to be recorded in tables and then graphed.

Analysis of results

1 Which test tube in Part A was the most effective at reducing heat loss? Suggest what makes this set-up most effective at reducing heat loss.
2 Which test tube in Part B was the most effective at increasing heat loss?

Discussion

1 What structural feature of mammals is the cotton wool simulating?
2 How can an insulating layer of air be achieved in mammals?
3 How can the results from test tube B be used to explain the observation that a cat looks larger on colder days?
4 Based on the results, suggest why an individual feels cooler on a hot windy day compared with on a hot still day.

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5 Using the observations collected in this experiment, explain why panting in dogs is an effective way of losing body heat.
6 Why are animals like frogs at greater risk of perishing on a hot windy day? Use the experimental results to support your answer.

Taking it further

1 Which part of the experiment modelled the role of perspiration in maintaining body temperature?
2 Were any experimental controls used in Part A and Part B of this experiment? If so, explain what these were and discuss their importance.
3 Draw a diagram of a negative feedback model, using the examples of thermoregulation investigated in this experiment. Are all components of a feedback model completely demonstrated in this experimental set-up? Explain your answer.
4 When the body temperature in mammals starts to drop, a number of things happen. Describe some of these physiological and behavioural responses. Are any of these responses being modelled in this experimental set-up? Explain.
5 When the body temperature in mammals starts to increase, different physiological and behavioural responses occur. Describe these responses. Are any of these responses being modelled in this experimental set-up? Explain.

Extension

1 Devise a procedure for testing the effects of shivering on heat regulation. Use a procedure similar to the one in this experiment.
2 Explain why a person shivers during a fever, even though their body temperature is above 37°C.
3 Why would a small mammal shiver more than a large mammal on a cold day?
4 A small mammal was found to eat more than its body weight in food in a 24-hour period. A larger mammal ate less than its body weight in food in the same time period. Explain why.

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WS
Chapter 11 Activity sheet

CHAPTER 11 SUMMARY

- Water is essential to life and is known as the universal solvent. It plays a major role in the dissociation (dissolving and separation) of salts into their ions for metabolic activity.
- When a solution outside a cell is compared with one inside, it can be isotonic (equal concentration, equal water movement), hypertonic (more concentrated outside the membrane, water moves out) or hypotonic (more concentrated inside the membrane, water moves in).
- Organisms can have a number of structural features or behavioural and physiological responses that enable them to maintain water balance (osmoregulation).
- There are three types of nitrogenous waste: ammonia, urea and uric acid.
- The type of nitrogenous waste produced by animals is related to the amount of water in their environment. However, all mammals excrete urea (even aquatic mammals such as whales), due to shared evolutionary history.
- Ammonia is highly soluble in water and is highly toxic.
- Urea is moderately soluble in water. It is moderately toxic, but much less toxic than ammonia.
- Uric acid is insoluble in water and almost non-toxic.
- Kidneys play a major role in osmoregulation and in vertebrates have adaptations for the removal of nitrogenous waste.
- Nephrons filter the blood by filtration and reabsorption. The Bowman's capsule surrounds the glomerulus, which is the site of filtration. The proximal and distal tubules, the loop of Henle, and the collecting ducts are the sites of reabsorption.
- The hypothalamus and pituitary gland regulate reabsorption by increasing or decreasing the production of ADH. Increased ADH increases water reabsorption, which decreases water loss through urination.
- Animals have a variety of behavioural, physiological and structural adaptations for osmoregulation or osmoconformation.
- To maintain water balance and allow for gas exchange, some plants have developed specialised structural and physiological adaptations.
- Xerophytes are plants that are adapted to survive in arid environments.
- Halophytes are plants that are adapted to survive in environments with high salinity.

CHAPTER 11 GLOSSARY

Adhesion The attractive force between water molecules and the inner walls of a vessel (e.g. the xylem vessels)

Ammonia The direct product of the breakdown of protein or nucleic acids; it is extremely toxic and highly soluble in water

Antidiuretic hormone (ADH) A hormone that regulates the level of water reabsorption in the collecting duct of a kidney's nephron

Arid Describes an environment characterised by a severe lack of available water that hinders the growth of most plant and animal life

Bowman's capsule A cup-shaped structure found at one end of each nephron, in the cortex of the kidney; it surrounds a group of capillaries called a glomerulus

Capillary action The movement of water within the spaces of a porous material or a narrow tube due to the forces of adhesion and cohesion

Cohesion The attractive force between water molecules

Coordinating centre (modulator) A tissue or organ that receives messages from receptors (via sensory neurons) and coordinates a

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response, then sends the information to an effector via motor neurons; it is usually the hypothalamus

Estuary A transitional region in which fresh water from a river meets salt water from the sea

Excretion The removal of nitrogenous waste; in mammals, the nitrogenous waste urea is removed in a mixture known as urine

Filtrate The mixture that is filtered out of the blood and enters the capsule; it flows along the proximal tubule to the loop of Henle and then to the distal tubule and the collecting ducts, from where it flows into the ureter

Filtration A process that starts in the glomerulus, where fluid and solutes are filtered out of the blood to form a glomerular filtrate

Glomerulus A group of capillaries surrounded by a Bowman's capsule within a nephron; blood is filtered from the glomerulus and through the surrounding Bowman's capsule

Guard cells A pair of cells that surround a stoma, which opens or closes depending on environmental conditions, giving the plant some control over water loss. When they are turgid the stomata open, and when they are flaccid the stomata close

Halophyte A plant adapted to live in environments with high soil salinity (i.e. a high salt concentration), such as salt marshes and the mud flats of estuaries

Hormone A chemical messenger secreted directly into the bloodstream, other body fluids, or adjacent tissues, where it moves to its target cells

Hypertonic At a higher concentration than another solution. When a cell is surrounded by a hypertonic solution, water moves out of the cell via osmosis to dilute the surroundings, so the cell shrinks

Hypothalamus A small region of the brain that plays a major role in detecting and coordinating the response to a change in e.g. temperature or water content in the blood

Hypotonic At a lower concentration than another solution. When a cell is surrounded by a hypotonic solution, water moves into the cell via osmosis to dilute the cell, so the cell swells. (Animal cells, which have no cell wall, sometimes burst.)

Isotonic At the same concentration as another solution. If a cell and its surrounding solution are isotonic, there is no net movement of water between them and the cell maintains a constant volume

Metabolism The sum of all the chemical reactions occurring within an organism to maintain life; it includes reactions enabling an organism's growth, homeostasis and reproduction

Modulator (See **coordinating centre**)

Nephron The basic structural and functional unit of the kidney that filters the blood in order to regulate chemical concentrations, produce urine and eliminate nitrogenous waste

Nitrogenous waste The nitrogen-containing metabolic waste products of the breakdown of proteins and nucleic acids. Initially, ammonia (which is highly toxic) is formed. Many animals convert ammonia into a less toxic form – either urea or uric acid

Osmoconformer An organism in which the internal solute concentration changes with the concentration of solutes in the external environment

Osmoreceptor A receptor cell that detects changes in blood water content (osmotic pressure)

Osmoregulation The active regulation of an organism's water content; it maintains the fluid balance (water gain and loss) and the concentration of electrolytes (salts in solution) to keep internal fluids from becoming too diluted or too concentrated

Osmoregulator An organism that has specialised mechanisms for regulating internal water and solute concentrations, despite concentration changes in the external environment

Osmosis The passive diffusion of water across a membrane in response to a concentration

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gradient (osmotic pressure) caused by an imbalance of molecules on either side of the membrane

Phloem A type of vascular tissue (tubes) within which sugars and other dissolved organic substances are transported in a vascular plant

Reabsorption The process of substances in the filtrate being absorbed back into the blood; control of reabsorption enables the regulation of water and ions

Root pressure A force pushing on the water in the xylem, resulting from the active transport of salt ions into the root hairs, which causes osmosis to occur and water to move from the soil into the root hairs; root pressure is one of the forces involved in the transpiration stream

Salt bladder A modified epidermal hair (found in some halophytes) that accumulates salt; it can be discarded to reduce the amount of salt in the plant

Solute A substance, such as a salt, that is dissolved in a solvent

Solvent A substance in which another substance (known as a **solute**) dissolves

Stomata (singular stoma) Small pores, usually found on the underside of leaves, through which gases are exchanged in plants

Transpiration The evaporative loss of water (in the form of water vapour) from plants, usually through small pores called stomata (singular: stoma) found on the surface of a plant, mostly on the underside of leaves. The evaporation and diffusion out of the stoma occurs because of the concentration gradient in the water vapour between the inside and outside of the leaf. Water vapour moves down the concentration gradient, from an area of high water content to an area of low water content. The transport of water through the plant results in water loss.

Transpiration pull The set of forces that pull water from the roots to the leaves, including cohesion and adhesion; as water evaporates from the leaves, columns of water are drawn up through the xylem vessels

Transpiration stream The continuous flow of water from the roots to the leaves via xylem vessels due to the forces of cohesion, adhesion and root pressure

Turgid Describes a cell into which water has diffused, so that the walls are stretched and the cell is fairly rigid

Turgor A measure of the force that results from the water pressure inside plant cells; it is maintained by osmosis

Urea A nitrogenous waste formed from the breakdown of proteins and nucleic acids (nitrogen-containing compounds); it is less toxic than ammonia and moderately soluble in water

Uric acid A nitrogenous waste formed from the breakdown of proteins and nucleic acids (nitrogen-containing compounds); it is the least toxic and insoluble in water

Vascular plant A plant with a transport system consisting of xylem and phloem

Xerophyte A plant that has adapted to live in arid environments; it has developed specialised features that minimise water loss, while maintaining gas exchange

Xylem A form of vascular tissue that contains tubes, within which water is transported from roots to leaves in a vascular plant

CHAPTER 11 REVIEW QUESTIONS

Remembering

1. Name the effector in a negative feedback loop when there is an increase in the water content of the blood.
2. List three gases that are involved in gas exchange through stomata.
3. Identify the hormone that can change the permeability of the walls of the collecting ducts in kidney nephrons.
4. Tupong fish, small crustaceans and phytoplankton live in estuaries. Explain, in general terms, the mechanisms you would expect each organism to have for maintaining water balance.

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5 State a mechanism that mammals use when responding to a decrease in the water content of blood.

6 Draw and annotate a diagram to show the operation of a sunken stoma in a xerophyte plant.

Understanding

7 Explain what would happen to the water balance of a marine fish if it were placed in fresh water.

8 Name an animal that lives in the Australian desert, and describe one physiological adaptation it uses for water balance.

9 Figure 11.28 shows three different nephron structures, with varying loop of Henle length. Justifying your choice, indicate which of these nephrons would be found in:

- **a** a terrestrial mammal
- **b** a freshwater fish
- **c** a reptile.

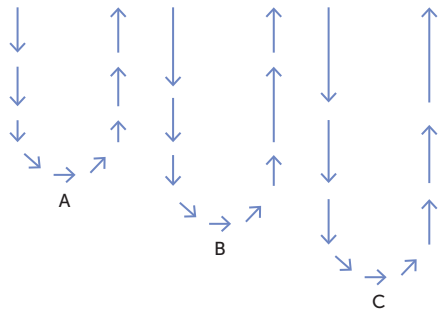

FIGURE 11.28 Loops of Henle from different organisms

10 Name the parts of the nephron, based on the described functions.

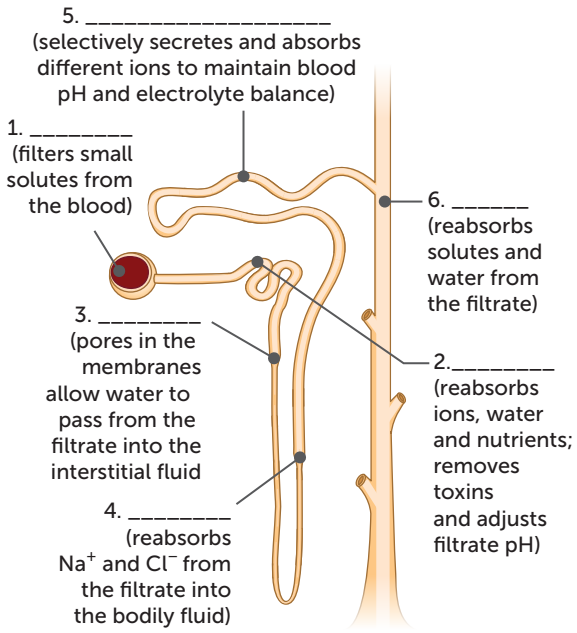

FIGURE 11.29 Nephron structures and their functions

Applying

11 Define metabolic activity and discuss how the ability to slow metabolic rate can assist some animals in maintaining water balance in an arid environment.

Analysing

12 Table 11.5 shows the filtrate volume in the various parts of a nephron.

TABLE 11.5 Changes in filtrate volume along a nephron

LOCATION IN NEPHRON	VOLUME OF FLUID (LITRES) PER DAY
Bowman's capsule	180
End of proximal tubule	54
End of loop of Henle	18
End of collecting duct (final urine)	1.5

If the measurement in the Bowman's capsule was assumed to be 100% of the filtrate, calculate the percentage of the filtrate that is retained as the final urine.

13 Outline the reason why the volume of filtrate becomes less as it flows through the nephron (see Table 11.5).

Evaluating

14 Some scientists have shown an interest in incorporating halophyte adaptations in future crops. Apply your knowledge of these adaptations to decide whether it is worth funding research into this area.

Creating

15 Draw three diagrams showing the three types of solutions (hypertonic, hypotonic and isotonic) surrounding red blood cells (animal cells). Show the direction of water movement for each cell.

Reflecting

16 Recall the different adaptations employed by plants and animals for water balance. Decide whether plants or animals have a better set of adaptations and what the criteria would be for your decision? Would you prefer to be a plant or an animal living in an arid or saline environment? Explain your answer.

PRACTICE EXAM QUESTIONS

1 When the cells from a plant root are placed in solution, they lose water to the solution. Relative to the cells, the solution is:

- **A** hypertonic
- **B** hypotonic
- **C** isotonic
- **D** osmotic.

[Q3 2019 SCSA]

2 Select the correct statement.

- **A** All mammals excrete nitrogenous waste in the form of urine.
- **B** All mammals excrete nitrogenous waste in the form of urea.
- **C** Aquatic mammals excrete ammonia and land mammals excrete urine.

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D Desert mammals excrete uric acid and other mammals excrete urea.

[Q9 2019 SCSA]

3 Freshwater bony fish mainly:

A gain salts by active transport through the skin

B gain salts by active transport through the gills

C lose salts by osmosis through the skin

D lose salts by osmosis through the gills.

[Q25 2019 SCSA]

4 A biologist conducted an experiment to test the ability of four species of small mammal to produce concentrated urine during periods of water shortage. The biologist measured the concentration of salt in the urine and blood in dehydrated individuals of each species. The results were expressed as the ratio of the concentration of salt in the urine to the concentration of salt in the blood (U:B ratio) and are given in Table 11.6.

TABLE 11.6 Ability of small mammal species to concentrate urine during water shortage

SPECIES	U:B RATIO
A	8:1
B	9.5:1
C	10:1
D	16:1

From the results, which species is most likely to inhabit a dry environment?

A A

B B

C C

D D

[Q29 2018 SCSA]

5 A marine fish regulates its water and salt balance. Is this an example of homeostasis? Give reasons for your answer. (3 marks)

[Q33b 2019 SCSA]

6 List four structures seen in the cross-section of a xerophyte's leaf that would assist the plant to conserve water. (4 marks)

[Q33c 2019 SCSA]

7 The root systems of xerophytes often include spreading roots just beneath the soil surface. Outline two advantages of these surface roots for xerophytes. (4 marks)

[Q33d 2019 SCSA]

8 Vertebrates produce three main types of nitrogenous waste.

[Q31b, c, d, e 2018 SCSA]

a Copy and complete the table to indicate the type of nitrogenous waste excreted by each animal. (4 marks)

ANIMAL	TYPE OF NITROGENOUS WASTE
Desert rat	
Bony fish	
Insect-eating bird	
River dolphin	

b Which type of nitrogenous waste is the most toxic? (1 mark)

c List the main types of nitrogenous waste in order from the one that takes the least amount of energy to produce to the one that takes the most energy. (3 marks)

d Describe the circumstances in which it is an advantage to an animal to excrete uric acid. (4 marks)

e Marine bony fish excrete only a small volume of urine. Explain why. (4 marks)

9 Discuss how a xerophyte minimises water loss while maintaining gas exchange. (10 marks)

[Q39b 2018 SCSA]

12 INFECTIOUS DISEASES

CHAPTER 12 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 What is a pathogen? What effect do pathogens have on organisms?
2 Are all pathogens the same?
3 What structural features of pathogens cause virulence?

SCIENCE UNDERSTANDING

» infectious disease differs from other disease in that it is caused by invasion by a pathogen and can be transmitted from one host to another
» zoonoses, such as influenza, can be transmitted between vertebrate species
» the major groups of organisms that cause disease are bacteria, fungi, protists and viruses; each group can be distinguished by its structural characteristics
» diseases caused by these major pathogen groups include
  – tuberculosis, tetanus, crown gall of plants
  – chytridiomycosis (amphibian chytrid fungus disease)
  – malaria, Phytophthora dieback (jarrah dieback)*
  – influenza, Ross River virus, viral diseases of honeybees, Australian bat lyssavirus

*The Phylum Oomycota containing Phytophthora dieback has been removed from the Fungi Kingdom and placed in the Protista Kingdom

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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12.1 WHAT IS AN INFECTIOUS DISEASE?

A disease is any condition that interferes with how an organism, or any part of it, functions. Diseases are described as **infectious** (**communicable**) if they are caused by an invasion by a **pathogen** and can be transmitted from one **host** to another. A host is an organism infected with a pathogen. An **infection** is occurring if a pathogen has entered a host, has established itself and is replicating. Unwanted signs and **symptoms** usually result from damage to the tissues and organs of the host. A **micro-organism** is not a pathogen (i.e. not pathogenic) unless it causes disease.

Humans have attempted to identify, prevent and manage infectious diseases for centuries. To identify the specific cause of an infectious disease, scientists have (and still apply) a series of postulates that were developed by Robert Koch in 1884.

Koch's postulates can be summarised into four steps.

1. The potential pathogen must always be present when the disease occurs.
2. The organism can be isolated from the host and grown in pure culture.
3. When organisms from the pure culture are inoculated into a healthy, susceptible host and the disease develops, this is further evidence for a specific cause.
4. The organism can then be re-isolated, grown in pure culture and compared with the organism first injected for confirmation.

Although Koch's postulates are limited because he only investigated bacterial pathogens, and some harmless bacteria may acquire extra **virulence** factors that make them pathogenic, his work provides the basis for identifying the specific cause of an infectious disease.

A pathogen is an **infectious agent** that causes disease. There are several different types of pathogens, but the four most common pathogen groups are **viruses**, **bacteria**, **fungi** and **protists**. This chapters describes 10 infectious diseases that are caused by these four types of pathogens.

Science Photo Library/Dr John Brackenbury

FIGURE 12.1 Short-duration flash photograph of a sneeze, showing the number of droplets expelled. Each droplet may contain thousands of bacterial or viral pathogens.

Table 12.1 lists the 10 infectious diseases that are listed in the Biology ATAR Syllabus [SCSA]. Viral pathogens cause influenza, honeybee diseases, Australian bat lyssavirus and Ross River virus. Bacterial pathogens cause tuberculosis (TB), tetanus and crown **gall** of plants. A fungal pathogen causes chytridiomycosis (amphibian chytrid fungus disease). Protists are the disease-causing agents of malaria and phytophthora dieback (jarrah dieback). You will learn more about these ten pathogens and the diseases they cause in this chapter, as well as their life cycles (chapter 13) and management strategies (chapter 14).

TABLE 12.1 Ten infectious diseases and the types of pathogens that cause them

VIRAL	BACTERIAL	FUNGAL	PROTIST
• influenza • Ross River virus disease • viral diseases of honeybees • Australian bat lyssavirus	• tuberculosis (TB) • tetanus • crown gall of plants	• chytridiomycosis (amphibian chytrid fungus disease)	• malaria • phytophthora dieback (jarrah dieback)

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Transmission is the passing of an infectious disease from an infected host to another individual. Pathogens have a variety of adaptations that enable transmission from host to host in a number of ways. Infectious diseases, such as TB, are caused by an agent that can be passed from an infected host to a susceptible (future) host. Diseases that are easily transmitted by close contact with an infected organism or their secretions (**body fluids**) are called **contagious**. A disease can be infectious but not contagious, as is tetanus.

Zoonotic diseases are infectious diseases that can be transmitted from one vertebrate group to another. Humans, for example, can be infected with avian (bird) or swine (pig) influenza viruses. Transmission is primarily through *direct contact* with infected animals. Direct contact with an infected host's saliva, mucus, faeces, blood or urine may happen when handling birds or by being bitten or scratched. Transmission may also happen through *close contact*, such as being near an infected bird when they shake their feathers. The virus may become airborne and be inhaled. *Indirect contact* may occur when a susceptible host comes into contact with areas where infected animals live or roam, where surfaces or objects have been contaminated. Examples of contaminated materials include chicken coops, pet food dishes and soil.

Due to globalisation, **outbreaks** of diseases in Hong Kong in 1997 [avian influenza A(H5N1) virus] and in China in 2003 [severe acute respiratory syndrome coronavirus (SARS-CoV-1)] resulted in influenza viruses spreading from Asia to Europe and Africa. The COVID-19 pandemic was first identified in China in 2019 and quickly spread around the world. The virus responsible, SARS-CoV-2, is a new zoonotic RNA virus. The COVID-19 pandemic is discussed in more detail in Chapter 14. Zoonotic influenza infection in humans may cause fever, cough or rapid progression to severe pneumonia. Prevention of zoonotic diseases is recommended and can involve simple physical strategies such as washing your hands with antimicrobial handwash to remove or kill the pathogens. The dynamics of zoonotic disease transmission are deeply embedded in the ecology and evolutionary biology of their hosts. A zoonosis comprises interaction between at least three species: one pathogen and two host species (an animal species acting as the **reservoir** of the infection, and humans).

Other zoonotic diseases explored in this course are the Australian bat lyssavirus and Ross River virus disease. Transmission of Ross River virus disease involves a **vector** (vectors are discussed in Chapter 11).

Australian bat lyssavirus
Read how Australian bat lyssavrius can be transmitted by bites and scratches from infected bats.

Key concept

Infectious diseases are caused by a pathogen and can be passed from one organism to another. Infectious diseases can be caused by viruses, bacteria, fungi and protists.

SCIENTIFIC LITERACY

Protecting human and animal health – a WHO discussion

There has been a rise in emerging infectious diseases, particularly zoonotic diseases such as influenza. Factors that are affecting the spread of zoonotic diseases include changes in the environment, habitat destruction and global movement. The World Health Organization (WHO) predicted in 2019, prior to the emergence of COVID-19, that the next human pandemic was likely to be zoonotic.

Questions

1. In what way can changes in the environment or climate affect the spread of infectious disease?
2. In what way can habitat destruction affect the spread of infectious disease?
3. In what way can an increase in global movement of people and wildlife affect the spread of infectious disease?
4. Construct an inference about why zoonotic diseases are emerging faster than they did in previous decades.

Question set 12.1a

REMEMBERING

1 Define:
   - **a** infectious disease
   - **b** pathogen
   - **c** contagious.

2 List the four main groups of organisms that can cause infectious disease.

UNDERSTANDING

3 If a new infectious disease emerged and you were a scientist investigating the cause, describe the steps you would take to find out the cause.

4 Explain why influenza is classified as a zoonotic disease.

Understanding infectious disease

Most micro-organisms are not pathogens. The fact that a micro-organism is pathogenic is due to special characteristics of the organism. These include the ability to stick to or invade a particular cell type, produce toxins, and cope with or avoid the host immune system. Pathogens differ in their disease-causing capacity or **pathogenicity**. The intensity of the effect of the pathogen is called its virulence. The virulence of a micro-organism is a measure of the severity of the disease it causes. Virulence factors help a pathogen invade a host, cause disease and evade host defences.

Individuals vary in their **susceptibility** to a pathogen; some have greater **resistance** than others. For example, if a cold is spreading through family and friends, every person does not necessarily become ill. Almost certainly, every person in contact with the sufferer will have contact with the cold virus, but not everyone will develop cold symptoms. An individual's ability to avoid being affected by a pathogen depends on a number of factors, such as their age, state of health and their natural resistance to that particular pathogen. Transmission of infectious diseases, including zoonotic diseases, depends on three factors: the infectious agent, a susceptible host and a mode of transmission.

Symptoms are the effects the pathogen has on the body of the host. For example, an annoying cough and sore throat may be early signs of a TB infection. Diseases usually have characteristic symptoms, and a knowledge of symptoms is useful to doctors trying to diagnose the cause of a disease without actually isolating the pathogen itself.

For many pathogens, symptoms of the disease do not appear immediately upon infection. The time between infection and the onset of symptoms is known as the **incubation period**. This time lag (Figure 12.2) may occur for a number of reasons. For example, the pathogen may have to divide many times to reach numbers sufficient to cause disease, or it may take time to reach the target tissues that are susceptible to that particular pathogen. Toxins produced by bacteria as waste products of metabolic activity may take time to accumulate to a level that affects the host. Diseases are often contagious before the onset of symptoms. This means that the pathogen can be passed on before the person even knows they have it. This incubation period may be an adaptation of the pathogen, allowing it to be transmitted before the host is incapacitated by symptoms.

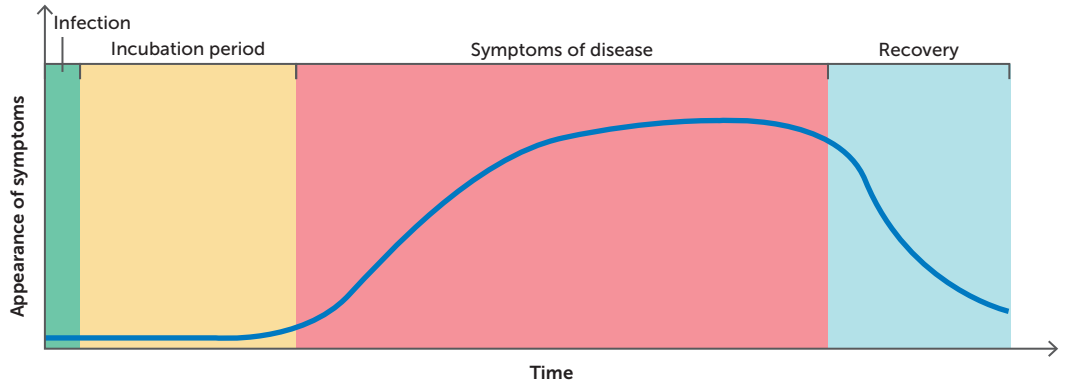

FIGURE 12.2 The phases of an infection and appearance of symptoms over time

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Key concept

Transmission of infectious diseases depends on three factors: the infectious agent, the susceptibility of the host and the mode of transmission.

Question set 12.1b

REMEMBERING

1 Define:
   a susceptibility
   b incubation period.
2 List three virulence factors that determine the pathogenicity of an organism.

UNDERSTANDING

3 Describe how doctors use symptoms to help diagnose and treat their patients.
4 Discuss the differences between pathogenicity and virulence.

12.2 NON-CELLULAR PATHOGENS

Viruses are non-cellular pathogens. Viruses consist of one or more strands of **nucleic acid** (RNA or DNA) inside a protein coat. They maintain this structure during the inert phase of their life cycle, that is, when they are not inside a host. Viruses are not made out of cells and therefore are non-living. They possess no metabolic machinery for processes such as cellular respiration. They cannot be classified as **prokaryotes** or eukaryotes. Instead of cellular features such as ribosomes and mitochondria, they have some nucleic acid and a protective coat. The shape of viruses varies greatly, but most viruses are relatively tiny compared with other pathogens. They are usually only viewed by electronic microscope.

Viruses

It is a common misconception that you can catch a cold if you go out on cold, wet days. The common cold is caused by a virus, not by becoming cold. When a virus infects an organism, it injects its nucleic acid into a host cell. Once inside, the viral nucleic acid takes over the host cell and directs it to make multiple copies of the viral protein coat and nucleic acid. These are then assembled into new viruses and are released when the host cell undergoes **lysis**, or splits open (during the '**lytic phase**'). This releases many more viral particles, which can infect other cells within the host. Exposure to cold and wet conditions might lower a person's resistance to the virus, but it is not the cause of the disease. All viruses cause some type of disease, because they rely totally on host cells for their reproduction. A virus is often referred to as an **obligate parasite** because it cannot function outside the host cell. This means that, unlike bacteria, viruses cannot be grown and studied outside live cells. This trait poses limitations on viral research.

Virtually every type of organism on Earth is susceptible to viral infection. Viruses are significant pathogens of many plants, sometimes resulting in the loss of crops such as potato and apples. Even bacteria have their own group of viral pathogens, known as **bacteriophages** (such as in Figure 12.4, page 411).

Structural features of viruses and virus replication

A virus is a non-cellular agent composed of a protein coat (**capsid**) and nucleic acid (Figure 12.3), either DNA or RNA, but never both. The infectious agent is microscopic, relatively small compared with all other pathogens and is usually measured at 30–300 nanometres in length.

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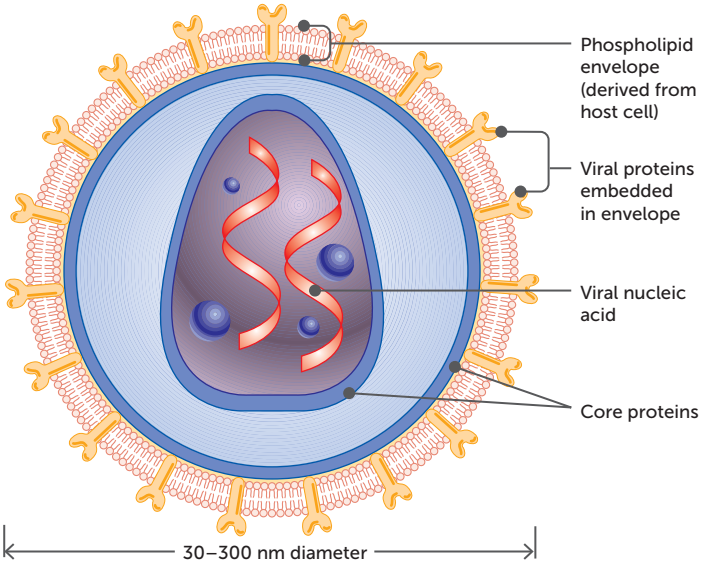
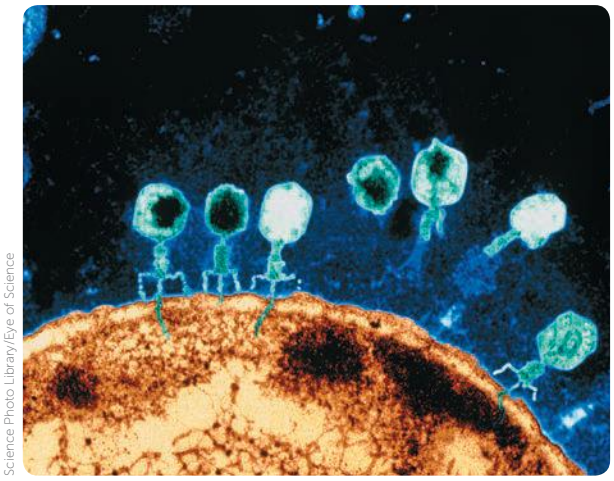


FIGURE 12.3 A virus consists of a nucleic acid core surrounded by a protein coat.



Science Photo Library/Eye of Science

FIGURE 12.4 A coloured transmission electron micrograph of viruses (blue) attacking a bacterial cell of *Escherichia coli* (orange). Small blue tails of DNA are seen being injected into the bacterium.

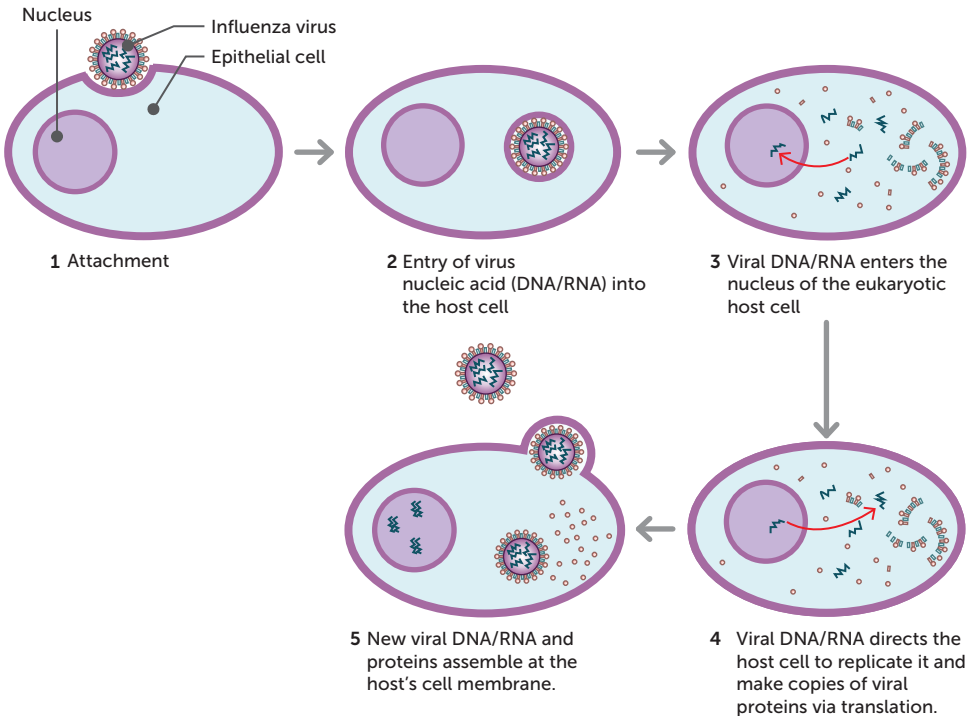


FIGURE 12.5 Steps in viral replication in a eukaryotic host

The life cycle of a virus
Watch the video and draw an annotated diagram representing each stage of the life cycle.

Each virus is usually limited to infecting a specific host cell or organism. For example, an adenovirus specifically infects epithelial cells in the upper respiratory tract, causing the common cold. This is because the virus is able to recognise and bind to **receptors** that are expressed only on respiratory tract epithelial cell surfaces. All viruses require host cells to replicate, and therefore all viruses are pathogenic. The host will experience symptoms when a virus is replicating inside the host's cells.

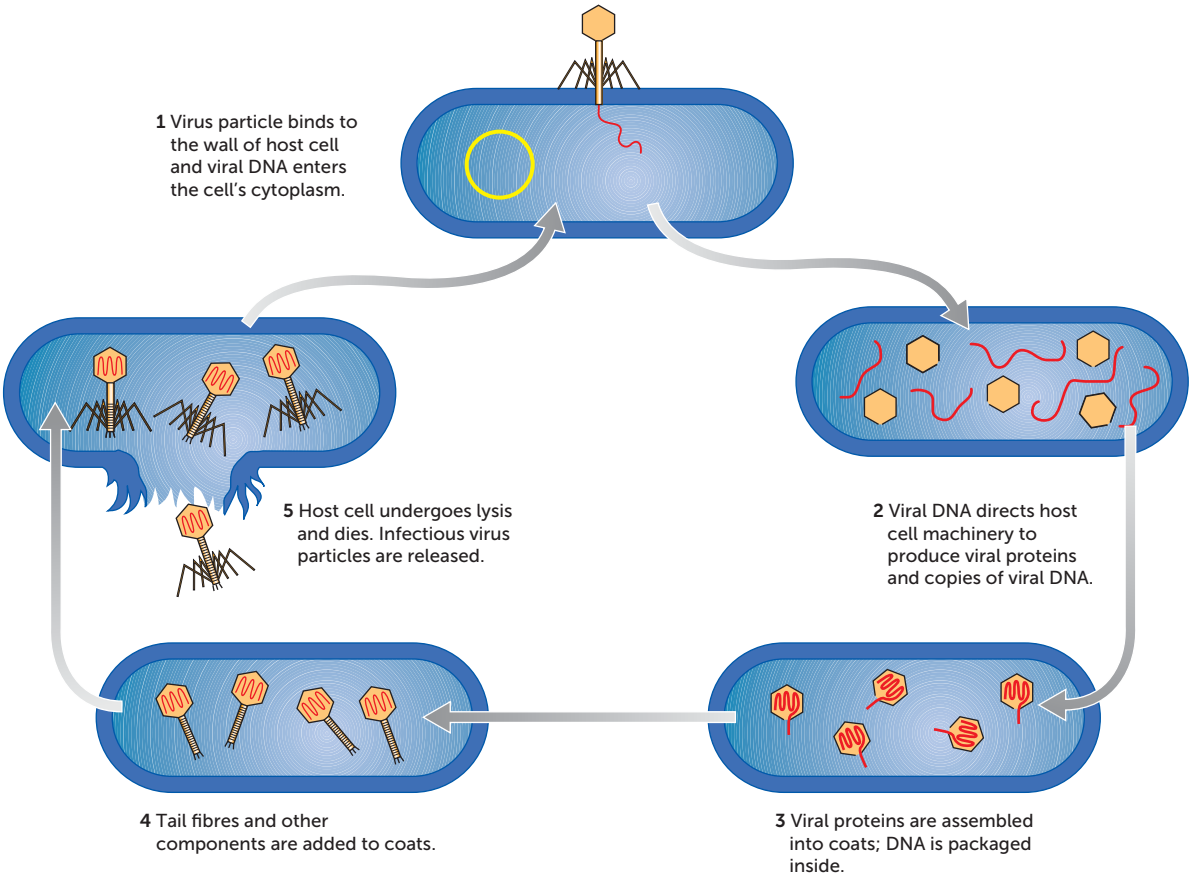
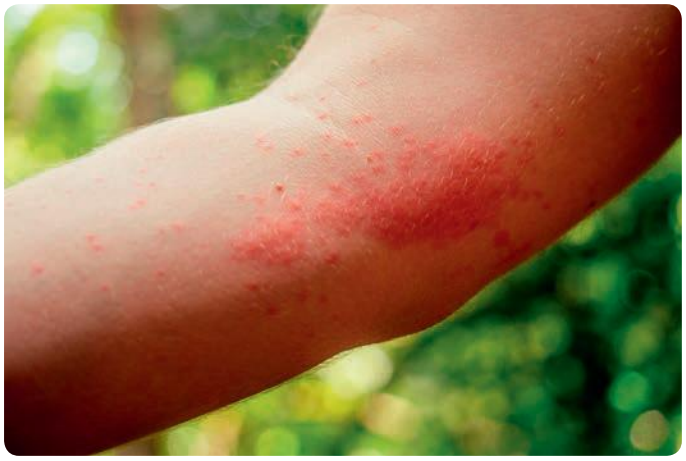


FIGURE 12.6 Viruses reproducing inside a bacterial (prokaryotic) cell. New viruses are produced within the infected bacterium.



Shutterstock.com/yurakrasil

FIGURE 12.7 This person is suffering a rash and joint pain, symptoms of Ross River virus disease. The primary replication of the virus occurs in skeletal muscle cells before the virus enters the blood. The virus also replicates in the mosquito vector.

Key concept

The structural features of viruses include that they:

- are non-cellular, not living
- have no membrane-bound organelles
- have nucleic acid (DNA or RNA)
- reproduce using host cells
- have a protective protein coat called a capsid
- are microscopic, 30–300 nanometres.

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TABLE 12.2 Diseases caused by viruses

NAME OF VIRUS	DISEASE	SYMPTOMS	INCUBATION PERIOD
Type A or B influenza virus	Influenza	The virus attacks the respiratory system. Sudden onset of high fever, cough, muscle aches and pains, sore throat, runny nose. These symptoms usually last for up to 2 weeks.	Average of 1–4 days
Ross River virus	Ross River virus disease	Rash on limbs or trunk for 5–10 days; painful and swollen joints, usually lasting for months; fever and headache.	1–3 weeks
There are many different honeybee viruses (e.g. chronic bee paralysis virus and deformed wing virus)	Viral diseases of honeybees [e.g. chronic bee paralysis virus (CBPV) and deformed wing virus (DWV)]	CBPV: trembling wings and body, failure to fly, loss of hair DWV: wing deformity but can be asymptomatic	Varies
Australian bat lyssavirus	Australian bat lyssavirus (ABL)	The virus attacks the nervous system: paralysis, delirium, convulsions/muscle spasms, death (if treatment is too late)	20 days to 27 months. Only three people in Australia have been infected and confirmed to have died from ABL.

Question set 12.2

REMEMBERING

1. List four diseases that are caused by viruses and four symptoms associated with each disease.
2. Define obligate parasite.
3. Recall the six steps a virus undertakes to replicate itself in a eukaryotic cell.

UNDERSTANDING

4. All viruses are pathogens. Justify this statement.
5. Describe a unique structural feature of a virus that distinguishes it from other cellular agents.
6. Viruses can be made of DNA or RNA. Explain the difference between DNA and RNA.
7. Viruses infect only specific host cells. Explain how this specificity comes about.

APPLYING

8. The Australian bat lyssavirus (ABL) and influenza pathogens attack different human systems. Explain how this relates to the type of symptoms experienced by the hosts.

12.3 CELLULAR PATHOGENS

All living organisms are made of cells and are characterised by the ability to grow, reproduce and respond to stimuli. In this section, we are not considering the vast majority of organisms on Earth; instead we are focusing on those few living organisms that cause disease: the pathogens. Cellular pathogens may be prokaryotic or eukaryotic.

Bacteria

Bacteria are prokaryotic. Bacteria are the most abundant and diverse group of organisms. Only a relatively small number of bacteria cause disease. There are billions of bacteria living on our skin and in our bodies that are not pathogenic and are often beneficial. Bacterium is the singular term for bacteria.

Bacteria: structural features and reproduction

Typically bacteria are **unicellular**, microscopic, 1–10 µm (micrometres) in length and 0.20–2 µm in diameter. Like all cells, bacteria have a plasma membrane that encloses the cytoplasm (Figure 12.8). As they are prokaryotes, they have no membrane-bound organelles (such as mitochondria) or a nucleus. However, bacteria do possess ribosomes and a single circular strand of DNA, known as a chromosome. In addition to the DNA found in the chromosome, bacteria contain **plasmids**. A plasmid is a small loop of DNA. Plasmids are able to be transferred out of bacteria without affecting the functioning of the bacteria. Some plasmids contain genes that act as advantageous alleles for the bacteria, as is the case for *Agrobacterium tumefaciens*, a bacteria that causes crown gall in plants. Most bacteria have a cell wall outside their plasma membrane, made of **peptidoglycan** (a protein–carbohydrate compound).

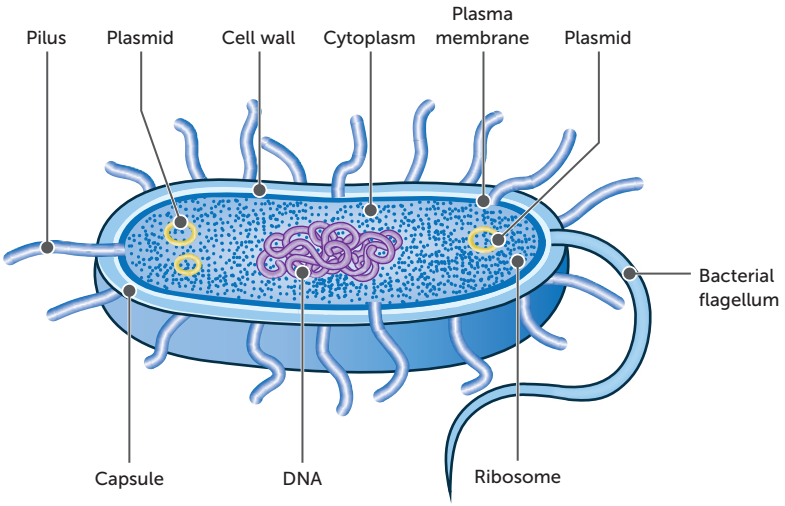

FIGURE 12.8 Generalised structure of a bacterium

Some bacteria possess a **flagellum**, which helps them to move about. Another adaptation found only in some species is a slimy **bacterial capsule**, which may be used to help the bacteria stick to surfaces, such as teeth or mucous membranes. The capsule is a thick, well-organised layer sitting outside the cell wall. It usually increases the virulence of a species, as it makes it harder for the body's immune system or antibiotics to attack the inner bacterium.

Many bacteria are capable of forming tough, dormant structures called **endospores** (or just **spores**), which are resistant to extreme temperatures, chemicals and drying out. This adaptation helps bacteria resist unfavourable conditions and facilitates dispersal to new hosts.

Some bacteria reproduce by **binary fission** (Figure 12.9), in which one cell splits into two identical daughter cells. Binary fission begins when the DNA of the bacterium doubles in quantity then divides into two (replicates). The bacterial cell then elongates and splits into two daughter cells, each with DNA that is identical to that of the parent cell.

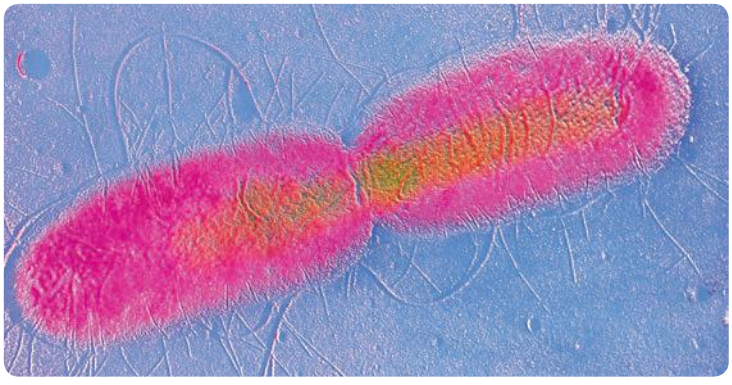

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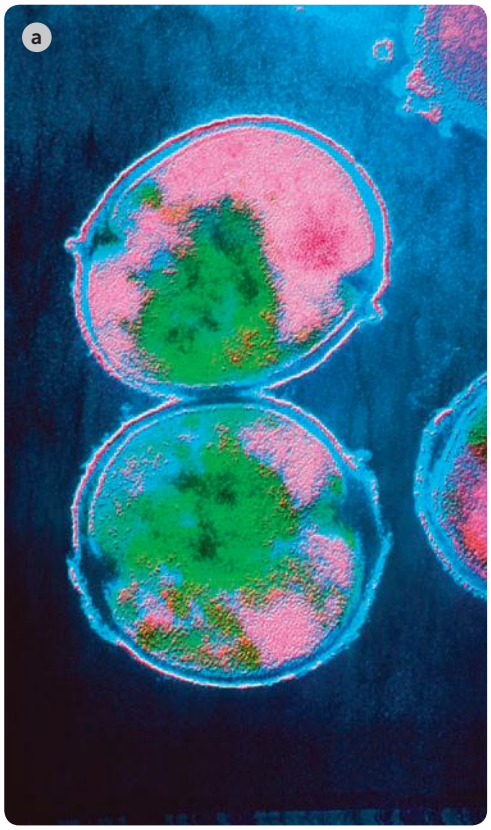
FIGURE 12.9 Transmission electron micrograph of *E. coli* dividing into two cells by binary fission

Others reproduce by budding off spores. These asexual forms of reproduction allow bacteria to reproduce very rapidly in favourable conditions. Some species can reproduce every 20 minutes. For such a species, one bacterium could give rise to a colony of 4.7×10^{21} individuals in just 24 hours. (That is 4 700 000 000 000 000 000 000 bacteria in a single colony!) *Mycobacterium tuberculosis*, however, has a much slower reproductive rate, taking 12 hours to divide.

Classification and identification of bacteria is related to their structure

To study bacteria in detail, it is necessary to view them under a powerful electron microscope. However, useful information can still be obtained by staining and using a light microscope. This reveals a variety of different shapes of bacteria. Bacteria can be classified according to their shape, namely:

- spherical, known as coccus (plural cocci) (Figure 12.10a)
- rod-shaped, known as bacillus (plural bacilli) (Figure 12.10b)
- spiral (plural spirilla) (Figure 12.10c)
- vibrio, rather like a comma.



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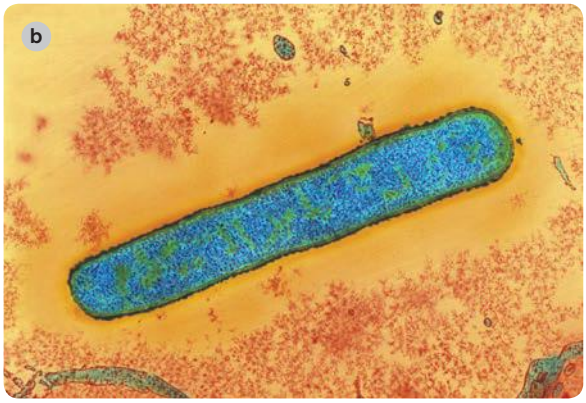

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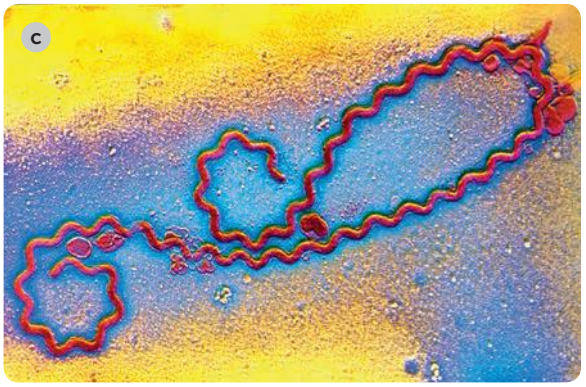

Science Photo Library/CNRI

FIGURE 12.10 a Transmission electron micrograph of a cocci-shaped bacterium, *Streptococcus pneumoniae* (magnification ×30 000); **b** a rod-shaped bacterium, *Bacillus anthracis*, which causes anthrax in sheep and cattle (magnification ×10 500); and **c** a spiral-shaped bacterium, *Leptospira* (magnification ×4400)

It is difficult to distinguish between the different strains of each shape. A pathogenic bacillus may look no different from a bacillus involved in cheese production. There is, however, one feature that can be a useful tool for classifying them. Many strains of bacteria have differences in the structure and composition of their cell walls, causing them to respond differently to stains and dyes, in particular, the Gram stain (Figure 12.11, page 416).

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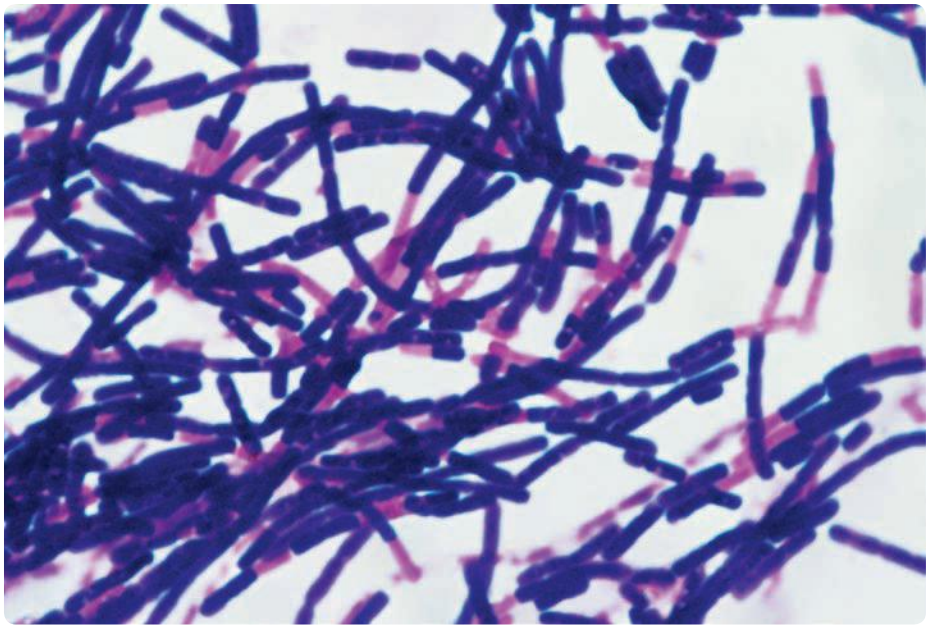

Science Photo Library/CNRI

FIGURE 12.11 Gram-positive bacteria stain purple and Gram-negative bacteria stain pink (magnification ×1000).

Since most bacteria are able to exist as free-living organisms, it is possible to grow colonies of them. This is done by inoculating a small number of a particular strain into a medium containing all their nutrient needs. This medium may be a liquid broth or a solid gel called agar (Figure 12.12). When one bacterium is inoculated onto a plate, it divides many times to form a visible colony. The appearance of these colonies can differ in colour, texture and shape, depending on the particular strain. An advantage of growing colonies on a solid medium is that individual strains can be isolated and grown in pure culture.

Science Photo Library/CC Studio

FIGURE 12.12 Haemolytic bacterial pathogens infect blood cells, so they must be grown on agar plates that contain blood.

Key concept

The structural features of bacteria include that they:

- are unicellular, prokaryotes
- have no membrane-bound organelles
- have circular DNA and plasmids
- may have flagella for movement
- reproduce via binary fission or budding off spores (endospores)
- are microscopic, 1–10 micrometres in length
- can be spherical, rod-shaped, spiral or vibrio
- vary in their ability to be stained (e.g. Gram stain).

How bacteria cause disease

Once inside a host, bacteria divide rapidly. Some bacteria damage host tissues directly, while others produce powerful **toxins** (often their own metabolic wastes) that disrupt the functioning of cells nearby or even further away. For example, toxins produced by the tetanus bacteria, *Clostridium tetani*, affect the nervous system of the infected human host. The pathogen is a spore-forming anaerobic bacterium. This means that outside of a host, the pathogen is in spore form and can survive in adverse conditions. As an anaerobic bacterium, the bacterial spores will only **germinate** and undergo asexual reproduction (binary fission) in the absence of oxygen, as in a deep puncture wound in human tissue. However, it is the toxins produced by the pathogen upon entry that travel along neurons and spread into the nervous system, inhibiting certain messages from being passed along the neurons and causing motor neurones to be hyperactive. This leads to severe, unopposed muscle spasms in the infected human host.

Bacteria are relatively large compared with viruses. Viruses are small enough to be taken up by host cells via receptor-mediated **endocytosis**. Instead of endocytosis, some bacteria can enter host cells via **phagocytosis**. This is a process performed by specialist white blood cells such as **macrophages**. Macrophages normally ingest then destroy foreign microbes.

TABLE 12.3 Bacterial diseases and their symptoms

NAME OF BACTERIA	DISEASE	SYMPTOMS	INCUBATION PERIOD
Clostridium tetani	Tetanus	Sustained, severe muscle contractions due to blocking of nerve impulses by tetanus toxin	3–21 days
Mycobacterium tuberculosis	Tuberculosis (TB)	A cough that persists, coughing up of sputum or blood, fever/night sweats, steady loss of weight, fatigue. These symptoms are observed in sufferers of active TB disease.	3–9 weeks from infection to development of a significant tuberculin. TB can stay dormant in the body for months or years. While TB is dormant, the host shows no symptoms. This is called 'latent TB'. The host is not infectious while it is latent.
Agrobacterium tumefaciens	Crown gall of plants	Galls form on stems, roots, trunks or branches, which can lead to stunted growth and wilting (because the gall formation interferes with water and food transport). Initially, galls form on the 'crown' of the plant, which refers to where the main roots join the stem, just above soil level.	8 weeks until galls become visible

Alamy Stock Photo/Nigel Cattlin

FIGURE 12.13 Close-up of a plant affected by crown gall

The disease TB is caused by the bacterium *Mycobacterium tuberculosis*. TB has affected the human race for thousands of years and remains one of the leading causes of mortality throughout the world. When a pathogen enters the respiratory system of a human host, macrophages in the lung's alveoli normally ingest and destroy the foreign microbes. Interestingly, some bacteria, such as *Mycobacterium tuberculosis*, have acquired the ability to survive, replicate and evade macrophages. The pathogen contains virulence factors that may increase the severity of the disease, especially if the host is susceptible (such as someone with a weak immune system). Instead of destroying the bacteria, the phagocyte provides a location where it can multiply through binary fission. While the bacteria is multiplying, and destroying host cells, the disease is categorised as active and symptoms develop.

Like the tetanus pathogen, the bacterium *Agrobacterium tumefaciens* enters its host through a wound. Unlike the tetanus pathogen, the host of *Agrobacterium tumefaciens* is a plant. This bacterium causes crown gall, a disease that involves the induced growth of tumour-like galls around the stem of plants. When the pathogen enters a wound, it inserts a gene from its plasmid into the genome of the host cell, causing rapid cell growth and the formation of galls. The galls are malformed growth that becomes a barrier in the infected host plant's transport system for water and nutrients, causing the plant to wilt and have stunted growth.

Binary fission
Play the animation to review binary fission.

Question set 12.3a

REMEMBERING

1. State three ways in which a bacterial pathogen can harm its host.
2. Define binary fission, and draw and annotate the following stages: replication begins at the 'origin of replication', a region of DNA on the chromosome and also on the plasmid; the two copies of the chromosome attach to the plasma membrane and there are now two copies of the plasmid; the cell elongates; a septum/cleavage furrow forms; cytokinesis occurs and the cell pinches in two; two identical daughter cells form, each possessing a plasma membrane and a cell wall.

UNDERSTANDING

3. Describe the advantages of bacteria:
   - **a** having a capsule
   - **b** forming endospores/spores.
4. Describe the methods by which different strains of bacteria can be classified or identified.

Fungi

The fungal world includes large organisms, such as mushrooms and toadstools, as well as minute forms that were only revealed with the invention of the microscope. These microscopic fungi include unicellular yeasts and moulds. They are plant-like organisms with cell walls, but their cell walls are not made of cellulose and the cells do not contain chlorophyll.

Structural features of fungi and fungi reproduction

Fungi are eukaryotes whose cells have membrane-bound organelles, including a membrane-bound nucleus. Fungal cells possess cell walls made of **chitin**, rather than cellulose. Most fungi do not have flagella. A flagellum is a long tail that, through whip-like motion, propels the spore form of fungi through water (a spore with a flagellum is called a **zoospore**). Chytrid fungi do have flagella and are therefore **motile** and able to move towards favourable conditions using the whip-like motion of the tail. Single-celled fungi are generally larger than bacteria. Most fungi grow multicellular **filaments**, structures that play an important role in how they obtain food. Filaments are long and thin, which gives them a high surface area for absorption. The network of tiny filaments that forms is called **hyphae (singular hypha)**. Hyphae have strong tubular cell walls (made of chitin) surrounding the cell membrane of each cell. Hyphae grow to form an interwoven

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mass known as a **mycelium**. This makes up the body of the fungus. A mycelium can infiltrate the tissues of the host on which it feeds. Most fungi produce spores, either through sexual or asexual reproduction. The mature mycelium forms **sporangia (singular sporangium)**, which release spores. When the spores make contact with a new, moist food source, they may germinate to form a new mycelium.

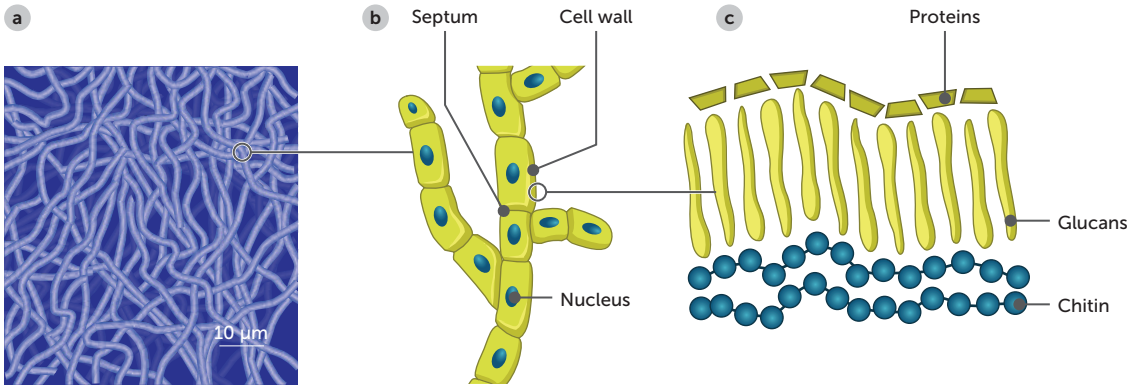

FIGURE 12.14 Basic structural features of fungi: **a** optical microscope image of a mycelium film showing a branched network of microfilaments (hyphae); **b** schematic representation of a hypha composed of cells separated by cross walls (septa), all enclosed within a cell wall; **c** schematic representation of the cell wall, a layer of chitin that surrounds the cell membrane

Key concept

The structural features of fungi include that they:

- are eukaryotic cell structure with membrane-bound organelles/nucleus
- have a cell wall made of chitin
- are unicellular or multicellular
- can be microscopic or macroscopic
- can be made up of filaments (hyphae)
- can have a body consisting of a mass of hyphae, known as the mycelium.

Some fungi are pathogenic, causing disease in a wide range of organisms, including plants and animals. As is the case with bacteria, not all fungi cause disease.

Most fungal diseases in animals are external, where they irritate and inflame the skin. A common example in frogs is chytridiomycosis (amphibian chytrid fungus disease). As a fungus grow on the skin, it produces spores. Spores are very long lived, an adaptation that improves transmission rates – they can remain alive for years, germinating when conditions are suitable.

TABLE 12.4 The fungal disease chytridiomycosis and its symptoms

DISEASE	FUNGUS	SYMPTOMS	INCUBATION PERIOD
Chytridiomycosis	*Batrachochytrium dendrobatidis*	Skin gets thickened and hardens. Respiration becomes difficult because significant gas exchange usually occurs across moist skin under normal conditions. The amphibian can become lethargic. Hind legs extend, and the amphibian becomes sluggish and has no appetite. These symptoms can lead to death.	2–10 weeks. Death follows the onset of symptoms within approximately 2–3 days.

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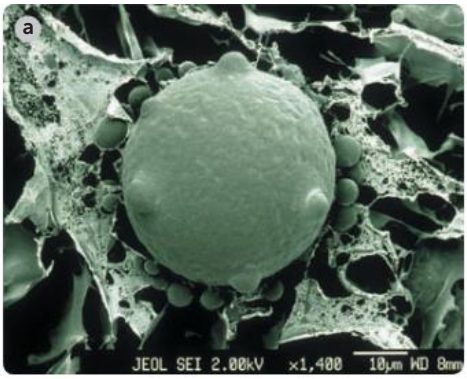

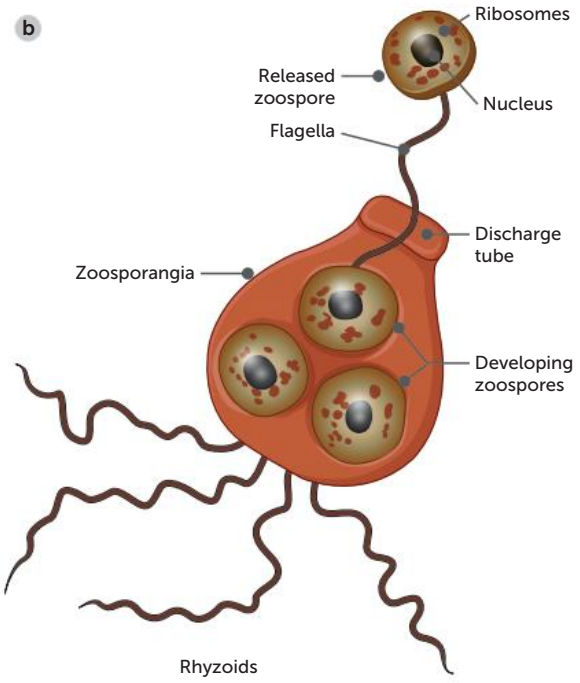

FIGURE 12.15 a Micrograph of the fungus pathogen *Batrachochytrium dendrobatidis* zoospores (×1400). **b** Diagram of the pathogen in its zoosporangia form with zoospores developing inside. **c** Amphibian displaying signs of chytridiomycosis (amphibian chytrid fungus disease), the disease caused by the pathogen.

CASE STUDY

History and ecology of chytridiomycosis

Batrachochytrium dendrobatidis live in water or soil. They produce spores that are motile in water, which means they can swim through water. Individual amphibians contract the disease when their skin comes into contact with water containing spores that have travelled from infected amphibians.

The chytrid fungus enters the surface layers of the frog's skin, causing damage to the outer keratin layer. When frogs are disease free, the skin has many functions, including gas exchange and regulation of osmosis and salts. As the skin hardens and thickens, these functions are hindered, along with homeostasis of water, salts and gases.

Susceptibility can vary. In some populations, mortality is 100%. The Australian Government has funded an abatement project, addressing the impacts of chytrid fungus. The two main goals are:

1 to prevent the spread of the fungus into areas in Australia that are disease free
2 to decrease the impact of the pathogen on populations currently infected.

Questions

1 Explain how susceptibility and mortality rates are related.
2 Explain why the rate of gas exchange decreases as the disease worsens. Describe the effect on frog cellular respiration.

Chytridiomycosis
Explore chytriodiomycosis by reading through this resource.

Protists

Protists are a diverse and mostly unicellular group of eukaryotic organisms. Of the 65 000 known species of protists, less than 24 species cause diseases in humans, but these few infect hundreds of millions of people each year. To date, we still do not have effective preventatives against many of them, and the treatment drugs we have are limited in their effectiveness. Some protists resemble animal cells, some resemble plant cells, and some resemble fungal cells. A variety of specific, unique features set them apart.

Structural features of protists and protist reproduction

Protists can be very small, smaller than prokaryotes. Their size can vary from 2 µm to 1000 µm (1 mm). They all have a membrane-bound nucleus and many are free-living. They can reproduce both sexually and asexually. The mode of reproduction can depend on the environmental conditions present. Protists are extremely diverse structurally, and their evolutionary history is complex. The paragraphs below describe examples of just two of the many groups of protists.

Species in the genus *Plasmodium* belong to a Protista group resembling animals. They have no chlorophyll or cell walls. Animal-like protists are sometimes referred to as protozoa. *Plasmodium* is classified into the parasitic group called Sporozoa (Apicomplexa). These parasitic protists spread through their host in the form of tiny infectious cells called **sporozoites**. Apicomplexans were given this name because at one end, the apex, the sporozoite cell contains specialised organelles for penetrating host cells. They have no means of locomotion.

Species in the genus *Phytophthora* belong to a group of Protista resembling plants. They have a cellulose-based cell wall. *Phytophthora* is classified into a group called stramenopiles because they have a distinguishable flagellum (plural flagella). A flagellum is a long, thin, thread-like organelle projecting from a cell. *Phytophthora* have a set of flagella (one hairy and one smooth) for locomotion, and they have an extensive network of filaments that allow for nutrient uptake. They are further classified as oomycetes (water moulds) because of their fungus-like network of filaments. *Phytophthora cinnamomi* parasitise terrestrial plants by attacking their root systems. Infected plants can be seriously affected, and *P. cinnamomi* is devastating jarrah forests in WA and Tasmania. The hyphae can penetrate the external surface of a plant and extend into its phloem, depriving it of valuable nutrients and reducing crop yield. Part of the pathogen's life cycle includes the production and release of large quantities of spores that effectively transmit these pathogens to new hosts. A spore is a reproductive cell that forms without fertilisation occurring. After germination, the spore can produce a mycelium, which is the new organism. *Phytophthora* spores are flagellated and therefore can be referred to as zoospores. Zoospores are only found in some protists and chytrid fungi.

What is malaria?
Read this interesting introduction to malaria.

The Malaria Challenge
Play the Malaria Challenge to learn about *Plasmodium*.

Malaria Lifecycle Part 1: Human Host
Watch the 4-minute video found here on malaria.

Phytophthora dieback
Read about phytophthora dieback and its impact in WA.

TABLE 12.5 Two protist diseases and their symptoms

DISEASE	PROTIST	SYMPTOMS	INCUBATION PERIOD
Malaria	*Plasmodium falciparum* is the most deadly. Five species of *Plasmodium* cause malaria.	Fever, headache, chills (shaking), sweating and vomiting. If left untreated and a host is susceptible, complications can develop, such as anaemia (because red blood cells burst) and liver failure. The complications can cause death.	10–15 days
Phytophthora dieback (jarrah dieback)	*Phytophthora cinnamomi*	Areas of the plant appear rotten and may have lesions (where the pathogen has consumed the cell's sugars). Wilting occurs, root systems die (dieback), and plant death follows (usually quickly and completely, not one branch at a time).	Depends on susceptibility of plant (affected by age and species). Incubation can range from weeks to months. Once wilting starts, death follows quickly.

More than one million Australians visit Bali every year. A protist of interest, *Giardia lamblia* (Figure 12.16, page 422), is a relatively common **parasite** that infects travellers to Bali. This flagellated protist can cause mild intestinal upsets, such as diarrhoea, but may also have more severe effects in the young or the elderly. It is often found in the bodies of cattle or wild animals and usually leaves their bodies (in the form of a cyst) through the faeces. People become infected if they drink water that contains these cysts. In Australia, this has not usually been a problem, because our sewage system is well isolated from our drinking water. However, it is a major problem in many developing countries, where travellers are advised never to drink water that is not bottled or boiled.

Protists and fungi
View this video on protists and fungi to reinforce your knowledge of their structures.

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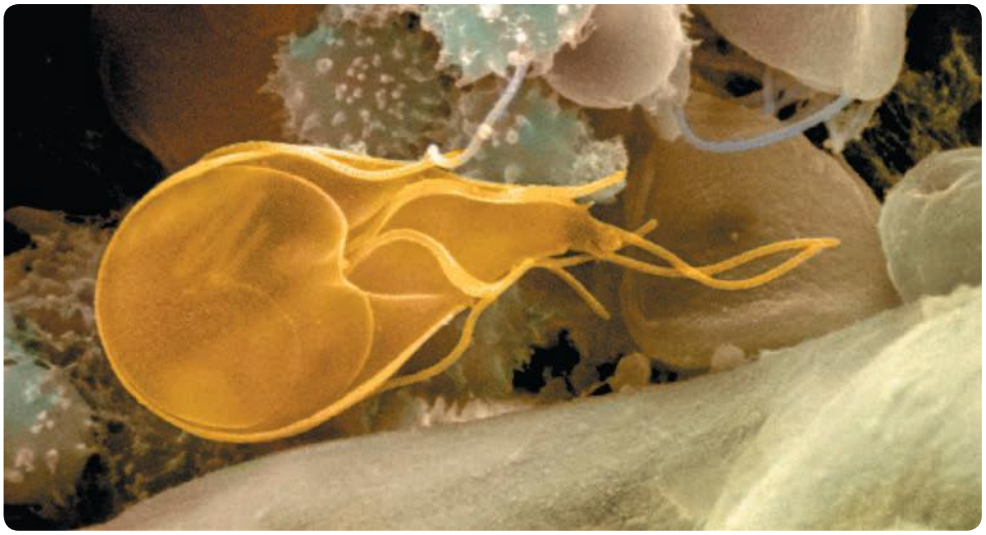

FIGURE 12.16 Scanning electron micrograph of *Giardia lamblia* (yellow) in the human small intestine. This flagellated protist contaminates drinking water, causing intestinal upsets.

Key concept

The structural features of protists include that they:

- are relatively small 2–1000 μm
- are eukaryotes, with a membrane-bound nucleus
- are mostly unicellular
- can reproduce sexually and/or asexually
- can exist in different forms during their life cycle, depending on their classification (for example, spores or zoospores, filaments, hyphae and mycelia)
- can be plant-like, animal-like or fungi-like in their structural or reproductive features.

12.1 APPLICATION

Fungi have cell walls. *Phytophthora cinnamomi* was originally classified as a fungus. It has a cell wall. It has a life cycle and reproduces in a similar way to a fungus. The pathogen grows fine filaments, called hyphae. Similarly to a fungus, it produces spores.

Can you explain why it is currently classified as a protist instead of a fungus?

(Hint: *Phytophthora* cell walls are made of cellulose. Its similarity to fungi is considered to be a case of convergent evolution.)

Question set 12.3b

REMEMBERING

1. Recall the structural features eukaryotic pathogens have in common.
2. Name and describe a plant disease caused by a protist agent.
3. Describe the symptoms caused by *Plasmodium falciparum*.
4. Distinguish between the structural features of fungi and protists.

UNDERSTANDING

5. Discuss the relationship between type of pathogen and type of symptoms.
6. Distinguish between the features of a fungal pathogen and a bacterial pathogen.
7. Describe the difference between malaria and *Plasmodium*.

CHAPTER 12 ACTIVITY AND INVESTIGATIONS

What does a million look like?

12.1 ACTIVITY

You will need

- Small quantity of rice
- A balance

What to do

1. Weigh out 1 g of rice.
2. Count the grains.
3. Calculate the mass of rice that would provide one million grains.

What did you discover?

Were you surprised by what a million looks like?

Fomites and pathogens

12.1 INVESTIGATION

Some bacteria can survive for days or even weeks on surfaces such as handrails, chopping boards and bathroom sinks. In this investigation, you will test the degree of contamination of four different fomites, by swabbing the objects and counting the number of bacterial colonies that grow on agar plates.

Aim

To compare the degree of contamination of four different fomites

Materials

Class requires:

- Incubator set to 25°C

Each group requires:

- 4 nutrient agar plates
- Marking pen
- Unopened box of sterile cotton swabs
- Sticky tape
- Disinfectant solution

Risks

WHAT ARE THE RISKS IN DOING THIS EXPERIMENT?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately.
Disinfectants may damage clothes and cause skin irritation.	Wear gloves and lab coats.

Procedure

Note: to minimise contamination, wipe the bench down with bleach or alcohol before you start.

1. Choose four objects, such as a doorknob, chopping board or coin, that you think may be covered with bacteria. Write a hypothesis to predict the degree of contamination of your four different fomites.
2. Sample one of your objects by rubbing a sterile swab tip across its surface.

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3 Open the lid of the agar plate and, starting at the top, gently drag the swab in a zig-zag motion down and across the agar, taking care not to gouge the surface.
4 Replace the lid quickly, seal the plate with sticky tape and label it with your group's name and the name of the object.
5 Repeat steps 2 to 4 using your other fomites.
6 Place plates in an incubator at 25°C for 24–48 hours.
7 Ensure the bench is wiped down with bleach or alcohol and wash your hands thoroughly.
8 Devise a way of scoring the amount of bacterial growth on each plate (e.g. no coverage, partial coverage, complete coverage etc.).
9 The next day, do not open the plates. Use your scoring system to record the amount of bacterial growth on each of the plates.
10 Dispose of your plates as instructed by your teacher, ensure the bench is wiped down with bleach or alcohol, and wash your hands thoroughly.

Results

1 Record your results for each fomite in a suitable table.

Analysis of results

1 Which fomite grew more colonies? Why do you think this was the case?
2 Describe the pattern observed in the size and number of colonies in the streaking on each plate.

Discussion

1 Was there a control in this experiment? Explain why this is, or is not, important.
2 List four factors that you would need to control to make this a fair (valid) test.
3 Identify any possible limitations in the data by considering the sample size and measurement errors.
4 Write a conclusion, ensuring that you refer back to your hypothesis.

Thinking deeper

It is clear that inanimate objects and the hospital environment can become contaminated with dangerous pathogens, and that these organisms can persist for long periods of time if not eradicated. Many Gram-negative species, such as *Escherichia coli*, can survive on inanimate surfaces for months. Mycobacteria, including *Mycobacterium,* also survive for many months on surfaces. A few others, such as *Haemophilus influenzae* and *Vibrio cholerae*, however, persist only for days. Explain how fomites can be directly linked to patient infection.
In which areas and on which equipment would you expect most contamination to occur, and how could you mitigate the spread of pathogens?

Fomites
More information about fomites can be found here.

12.2 INVESTIGATION

Investigate the effectiveness of different anti-microbials on pathogen growth

Design your own similar investigation into the effectiveness of three different antiseptics/antibiotics/antimicrobials such as Dettol, honey, tea-tree oil.
Use four Petri dishes. One acts as a control without an antimicrobial.

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Chapter 12 Activity sheet

CHAPTER 12 SUMMARY

- Disease is any condition that interferes with the proper functioning of an organism.
- Infectious diseases are caused by any agent that can be transmitted from one organism to another.
- Pathogens are disease-causing agents. Cellular pathogens include bacteria, fungi, protists, endoparasites and ectoparasites. Viruses and prions are non-cellular infectious agents that are always pathogenic.
- Pathogens have adaptations to ease their entry into cells of vectors, intermediate hosts and the final host.
- Transmission of disease occurs through various means, including direct contact, close contact and indirect contact. Contact can be with body fluids, the air, contaminated food or water, or disease-specific vectors.
- Specific diseases are characterised by their virulence, incubation period and recognisable symptoms.
- People differ in their susceptibility to various diseases.
- Viruses and certain parasites are host specific. Zoonoses can be transmitted between vertebrate species.
- Pathogens have adaptations to facilitate their transmission between hosts. Examples of such adaptations include long-lasting resistant spores (or similar, which enable them to remain dormant outside a host), use of a vector, and ability to exist in water.

CHAPTER 12 GLOSSARY

Bacteria Microscopic unicellular organisms that do not have a nuclear membrane or membrane-bound organelles – they are prokaryotic

Bacterial capsule A slimy layer surrounding the cell wall of some species of bacteria

Bacteriophage A virus that invades bacteria

Binary fission The division of a cell into two cells without mitosis; a prokaryotic cell undergoes binary fission to form two identical daughter cells; a form of asexual reproduction

Body fluid Any liquid that comes from inside the body

Capsid The protective protein coat of a virus

Chitin The polysaccharide that is the main component of fungal cell walls and the exoskeletons of insects and other arthropods

Communicable Able to be communicated (transmitted) from one organism to another

Contagious Able to be transferred by direct contact

Endocytosis A process by which material can pass into a cell: the cell membrane folds inwards to form a small sac around the incoming material or may extend outwards for larger particles (in which case it is termed phagocytosis)

Endospore A tough, dormant structure formed by many species of bacteria to help them resist unfavourable conditions and disperse to new hosts

Filament A thread-like series of tubular cells connected end to end; each cell is surrounded by a cell wall; each filament is a hypha, and multiple filaments are called hyphae; a mass of interwoven filaments is called a mycelium

Flagellum A whip-like tail, which provides a zoospore and some other motile single cells with locomotion

Fungi A diverse kingdom of spore-producing, eukaryotic organisms; they have a cell wall made of chitin; they do not possess chloroplasts; they have a complex cell cycle, in which spores can develop into hyphae then grow into a mycelium; they can be unicellular, but are mostly multicellular

Gall A brown, roughened lump of undifferentiated tissue on the crown of a plant (where the roots meet the stem on a small plant, or where a branch meets the trunk of a tree); it looks tumour-like

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Germinate Grow and develop from a spore into hyphae and a new mycelium (in the case of fungi); from a seed into the first root and shoot (in the case of plants)

Host An organism that is infected by a pathogen

Hyphae (singular hypha) A network or branch of tiny filaments; a hypha is one of the filament threads

Incubation period The time between infection and the onset of symptoms

Infection The invasion of host by a pathogen, where it establishes itself and replicates

Infectious (communicable) Caused by an invading pathogen and able to be transmitted from one organism to another

Infectious agent A disease-causing agent that can be transmitted from one organism to another

Lysis The process of a cell bursting (verb: to lyse)

Lytic phase Part of the life cycle of a virus in which viral components are replicated and packaged to form new viruses that lyse the host cell

Macrophage A white blood cell that can perform phagocytosis on microbes such as pathogens, by engulfing them (**endocytosis**) and destroying them with the use of enzymes

Micro-organism A microscopic organism; for example, bacteria

Motile Able to move spontaneously without external force

Mycelium An interwoven mass of hyphae; it forms the body of a fungus

Nucleic acid The molecule (DNA or RNA) that forms the genetic code in an organism

Obligate parasite An organism that can only survive in another organism; it is 'obliged' to live in another organism

Outbreak A sudden, unexpected increase in the prevalence of a particular disease above the baseline level for that population; it could be a single case of a contagious disease in a small community

Parasite An organism that lives on or in its host for all or part of its life, causing harm to and gaining nutrition from the host

Pathogen A disease-causing agent

Pathogenicity The capacity of a pathogen to cause disease in a host

Peptidoglycan A protein–carbohydrate compound that forms the cell wall of bacteria

Phagocytosis The process of engulfing and destroying a microbe

Plasmid A small, circular piece of DNA, found in bacteria, that is able to replicate independently of the cell's chromosomes; engineered plasmids can carry antibiotic-resistance markers

Prokaryote A single-celled organism that lacks membrane-bound organelles such as a nucleus

Protist An organism in the Kingdom Protista that is eukaryotic but may have plant-, animal- or fungus-like features; a protist is usually unicellular and relatively tiny

Receptor In cell biology, a site on a cell membrane that receives a signal, or the site on a host cell where a virus may attach prior to endocytosis

Reservoir An organism (such as a wallaby) or habitat (such as soil) in which a pathogen can reside, and sometimes replicate, prior to entering a susceptible host; a reservoir is somewhere in which the pathogen does not go extinct

Resistance When an infectious agent or toxin is acting on a host, resistance is the ability to withstand any adverse effects; it describes the extent to which an organism is or is not affected by an agent such as a pathogen or chemical toxin

Sporangia (singular sporangium) A spore case in which asexual spores are formed

Spore A reproductive cell that forms without fertilisation. Spores can produce a mycelium after germination

Sporozoite The tiny, infectious cell form of a parasite (such as *Plasmodium*); it is often the infective agent that enters the host; it is a relatively immature form of a pathogen

Susceptibility The likelihood of developing a disease; if the susceptibility of an organism is high, its ability to resist the disease is low

Symptom A subjective experience felt by a patient, such as nausea and pain

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Toxin A waste product of bacteria and other microbes that is poisonous to a host

Transmission Transport of a pathogen from an infected host or a reservoir to a susceptible host

Unicellular Single-celled

Vector In reference to diseases, a vector is an agent that transmits pathogens from one host to another; in genetics, it refers to a vehicle used to transfer DNA sequences from one organism to another

Virulence A measure of the ability of a pathogen to cause severe disease within its host

Virus A non-cellular pathogenic agent, containing either DNA or RNA, that can only reproduce inside a living host cell

Zoonotic disease A disease that animals pass to humans; an infection that is naturally transmitted between other vertebrate animals and humans

Zoospore A spore with a flagellum; it is one of several forms of a fungal or protistan organism

CHAPTER 12 REVIEW QUESTIONS

Remembering

1 Recall four symptoms of:
   - a influenza
   - b Ross River virus disease
   - c Australian bat lyssavirus.

2 Name one honeybee disease caused by a virus and describe two to three symptoms.

3 Identify two adaptations that aid the entry and transmission of fungal pathogens into a new host.

4 State two important differences between a bacterium and a virus. Give two examples of diseases that are caused by each of these pathogens.

5 Recall four symptoms of:
   - a tuberculosis
   - b tetanus
   - c crown gall of plants.

6 State two diseases caused by each of the following pathogens: fungi and protists.

7 Describe the feature that helps classify *Phytophthora* as a protist and not a fungi.

Understanding

8 Explain why classifying protists is challenging.

9 Which human systems are infected by the tuberculosis and malaria pathogens?

10 Explain why the symptoms of tuberculosis and malaria are so different, based on your answer to Question 9.

11 Draw an annotated diagram of the process by which bacteria reproduce.

Applying

12 Differentiate between pathogenicity and virulence, and include examples of organisms that demonstrate these features.

13 Define endocytosis and describe how it is applied in virus replication and in macrophage phagocytosis of tuberculosis bacteria.

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Analysing and creating

14 Name two structural characteristics or reproductive strategies characteristic of each of the four pathogen types listed below. Which of these are unique to the pathogen type? Create a Venn diagram showing the similarities and differences in structure between two of the types of pathogens.

- **a** virus
- **b** bacteria
- **c** fungi
- **d** protist

15 Figure 12.17 shows the number of cattle infected with the pathogen causing BSE (bovine spongiform encephalopathy; also known as mad cow disease) in Britain for the years 1985–95. Since 1992, feedstuff containing sheep offal has been banned.

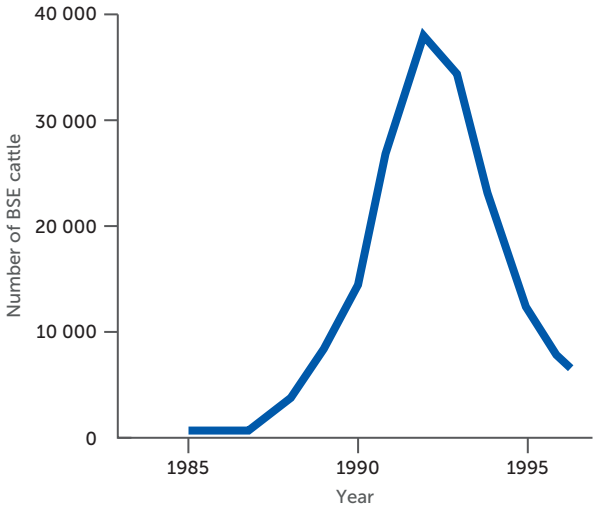

FIGURE 12.17 Number of cattle infected with BSE from 1985 to 1995

- **a** Describe the trend in numbers of BSE-infected cattle in Britain from 1985 to 1995.
- **b** Suggest a reason for the decline in the incidence of BSE since 1992.
- **c** The infectious agent that causes BSE can infect humans, causing Creutzfeldt–Jakob disease. Does this make Creutzfeldt–Jakob disease zoonotic? Explain your answer.

16 Consider the stages in the replication of a virus. Imagine you are a chemist trying to find antiviral medicines. Describe two points at which a virus would be susceptible to antiviral chemical therapies.

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PRACTICE EXAM QUESTIONS

1 Which of the following human diseases is transmitted by a vector?
- **A** influenza
- **B** tuberculosis
- **C** tetanus
- **D** malaria

[Q5 2017 SCSA]

2 Chytridiomycosis is a:
- **A** fungal disease of plants
- **B** fungal disease of amphibians
- **C** bacterial disease of plants
- **D** bacterial disease of amphibians.

[Q5 2018 SCSA]

3 Which of the following statements about fungal and bacterial cells is *most* accurate?
- **A** Neither fungal cells nor bacterial cells have cell walls.
- **B** Fungal cells have cell walls but bacterial cells do not.
- **C** Fungal cells do not have cell walls but bacterial cells do.
- **D** Both fungal and bacterial cells have cell walls.

[Q6 2018 SCSA]

4 In order to be regarded as infectious, a disease must be:
- **A** caused by a pathogen
- **B** caused by a mutation
- **C** able to infect humans
- **D** able to infect animals.

[Q13 2018 SCSA]

5 Crown gall disease in plants is caused by:
- **A** a bacterium that enters the plant through stomata in the leaves
- **B** a bacterium that enters the plant through wounds in the roots or stem
- **C** a virus that enters the plant through stomata in the leaves
- **D** a virus that enters the plant through wounds in the roots or stem.

[Q24 2018 SCSA]

6 Malaria and tuberculosis are infectious diseases of humans. Malaria is caused by a protist. Describe the main structural features of protists. (4 marks)

[Q35a 2018 SCSA]

7 There are four main groups of organisms that cause infectious disease. Protists are one of these groups. Name the three other groups and describe their structural characteristics. (10 marks)

[Q38a 2016 SCSA]

8 State how infectious disease differ from other types of disease. (2 marks)

[Q34a 2016 SCSA]

9 State the type of organism that causes the following diseases and the type of organism affected by the disease. An example is tuberculosis. Tuberculosis is caused by a bacterium and affects humans. (6 marks)
- **a** crown gall
- **b** chytridiomycosis
- **c** phytophthora dieback

[Q34b 2016 SCSA]

10 Name and describe the process by which a bacterial cell reproduces. (4 marks)

[Q31a 2016 SCSA]

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13 SPREAD OF PATHOGENS

CHAPTER 13 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Can you give examples of the different ways pathogens can spread from host to host?
2 Why are some pathogens spread more easily and transmitted further and faster than others?
3 How are some infectious diseases transmitted by mosquitoes?

SCIENCE UNDERSTANDING

» the life cycle of a pathogen and its associated diseases, including the method of invading the host, the impact on the host, and the mode of transmission (direct or indirect), determines its success for survival
» the spread of a specific disease involves a range of interrelated factors, including
  – growth of the pathogen population
  – density of the host population
  – mode of transmission
» transmission and spread of disease are facilitated by regional and global movement of organisms
» the distribution of mosquito-borne diseases may be affected by global climatic changes
» many pathogens evolve rapidly in a changing environment

SCIENCE AS A HUMAN ENDEAVOUR

» susceptibility of urban areas to epidemics and pandemics of infectious disease can be due to population density, variation in living conditions and healthcare provisions

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

Getty Images/Max shen

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13.1 THE LIFE CYCLE OF A PATHOGEN

As for all organisms, pathogens strive to survive and reproduce, but pathogens usually do so at the expense of a host. The host provides an environment that assists in the survival of the pathogen. In order to best survive and reproduce, pathogens need to:

- invade the host (have a **portal of entry**)
- exploit a nutrient-rich area of the host
- avoid host **defence mechanisms**
- replicate
- exit (have a **portal of exit**) and transmit to new hosts (mode of **transmission**).

This is known as a pathogen's **life cycle**. Some pathogens have developed elaborate life cycles that enable them to survive, reproduce and **spread**. Pathogens spread by being transmitted from an infected host (or **reservoir**) to a **susceptible host**. The infected host may travel and thus transport a disease, which further increases spread. Understanding the infectious cycle is critical for identifying suitable strategies for controlling a pathogen.

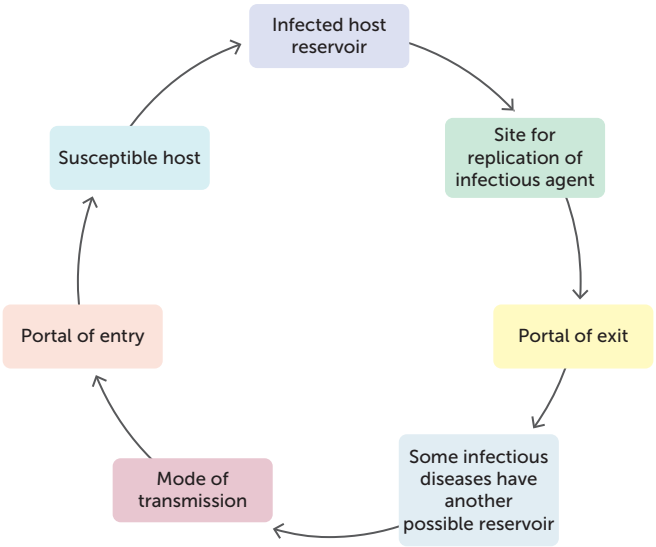

FIGURE 13.1 Generic life cycle of a pathogen

There are different ways pathogens can enter a host. The susceptible host has various portals of entry through which the micro-organism can enter. Portals of entry can be **mucous membranes**, which are surface membranes that are moistened with slimy, sticky and viscous mucus. Mucous membranes are found in the human respiratory, gastrointestinal and reproductive tracts. They are lined with epithelial cells that secrete mucus. Other portals of entry are skin, wounds, eyes and ears.

The outer layer of tissue in a plant or a human is known as the epidermis. It usually provides a physical barrier to pathogens. As long as it remains unbroken, the tough waterproof skin is an effective barrier against invaders; however, the external openings of the respiratory, digestive, excretory and reproductive systems provide ideal entry points into any organism. If the skin is wounded, a pathogen can penetrate the barrier using the wound as a portal of entry. Examples of pathogens that enter through wounds include the bacteria *Agrobacterium tumefaciens*, which causes the formation of a **gall** in the **crown** areas of plants, and *Clostridium tetani*, which causes tetanus in humans.

Many pathogens enter the host via the same portal that they exit: for example, the pathogen *Mycobacterium tuberculosis,* which causes the disease tuberculosis (TB). This bacteria enters and exits through the respiratory system. The infected host may cough, and the susceptible host may inhale the **airborne droplets** containing the pathogen.

TABLE 13.1 Portals of entry and exit

PORTALS OF ENTRY	PORTALS OF EXIT
Skin (bite, blood feed, penetration)	Bite/blood feed/saliva
Mucous membranes	Digestion (elimination)
Wounds in epidermal layers of animals and plants	Respiratory system (coughing, sneezing)
Eyes and ears	Blood contact
Respiratory system (nose/throat/lungs)	Reproductive system (e.g. some honeybee viruses passing from queen bee to worker bees)
Reproductive system	

After entry, many pathogens attach to host cells. Some pathogens then multiply in between host cells or within body fluids, while others, such as viruses and some bacteria, enter the host cells and replicate there.

The **impact on the host** can include mild to severe **symptoms** or **signs**. Many of these are listed in chapter 12. Tissue structure and function is usually affected. Tissue damage or abnormal function may be due to the replication of the pathogen, or toxins produced by the pathogen, and death is a possible outcome. Symptoms are usually subjective and include the feelings or experiences of a patient, such as nausea and pain. In contrast, signs of disease are usually objective and measurable and can be directly observed. Elevated body temperature and breathing rate are considered vital signs of disease.

Key concept

The pathogen life cycle is dependent on having portals of entry and exit to and from a susceptible host, the ability to replicate, and modes of transmission.

Question set 13.1

REMEMBERING

1. List the general steps in the life cycle of a pathogen.
2. List three openings in the skin that can allow the entry of pathogens.
3. Describe mucous membranes and recall where they are found in humans.

UNDERSTANDING

4. Differentiate between signs and symptoms of a disease.
5. Explain why a wound can be a portal of entry.

13.2 MODES OF TRANSMISSION

The life cycle of a pathogen requires a mode of transmission. Without a mode of transmission, the pathogen will die when the host dies. Transmission is how the pathogen is transferred from the host and/or a reservoir to a susceptible host, which can involve developmental stages occurring in the environment or in **vectors**. Vectors are living or non-living things that transport pathogens from the infected host to a susceptible host. Mosquito vectors transport pathogens through blood feeds. Bat vectors transport pathogens through bites and scratches. The different modes of transmission are commonly classified as direct or indirect. **Direct transmission** is the transfer of a pathogen from an infected host, or other reservoir, to a susceptible host by **direct contact** or **close contact**. **Indirect transmission** is the transfer of a pathogen from a reservoir to a host through vectors (inanimate vehicles or living intermediaries) or suspended air particles. Indirect transmission may require one or more steps. Pathogens rely on the hosts they infect for nutrients or a site for replication. A reservoir is an organism or habitat (such as soil) in which a pathogen can reside, and sometimes replicate, prior to entering a susceptible host. Some pathogens have a **definitive host** (a single host), within which the adult phase of the pathogen produces gametes, and which also acts as a reservoir, whereas other pathogens require a host and a separate reservoir (an **intermediate host**, such as another species of animal).

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Direct transmission

Direct contact

Direct contact involves the transmitting of a pathogen through physical touch between the infected host and a susceptible host via skin or body fluids. Body fluids are any liquids that come from inside the body, including sweat, tears, vomit, nasal secretions, blood, saliva, sexual fluids and urine.

Close contact

Pathogens can be transmitted via airborne droplets when there is close proximity (usually within 1.5 metres) between infected and susceptible host, particularly by sneezing, singing or coughing. When an individual coughs or sneezes, small droplets of mucus that may contain pathogens are ejected, and these can be inhaled by someone close by. A cough, a sneeze or singing can release millions of microbes into the air in droplets of mucus or saliva that are so small they can remain airborne for extended periods of time. If a droplet lands on the mucous membranes of a person's mouth, nose or eyes, they may catch the disease. Sometimes talking, singing or just breathing out is enough to allow pathogens to leave the host and become airborne in aerosols. Transmission by airborne droplets through close proximity is classified as direct, because transmission is by direct spray over a short distance (before the droplets fall to the ground) from an infected host's respiratory system into a susceptible host's respiratory system. TB and influenza can be spread in this way.

From a reservoir

Transmission can occur from a reservoir directly to a susceptible host. A reservoir is a living or non-living site in which a pathogen normally resides and possibly replicates. The pathogen may be dormant in this site. An example of a non-living reservoir is soil (the reservoir of tetanus bacteria).

In **zoonotic diseases**, animals act as reservoirs of human disease and transmit the infectious agent to humans through direct or indirect contact. In some cases, such as ABL, the disease also affects the animal; in others the animal is **asymptomatic**. The first step requires the pathogen to exit the body of its current host. It must then gain transport to a suitable new host, enter their body, establish itself in their tissues and finally ensure it is once again passed to a new host.

Indirect transmission

Living vectors

A living vector is usually a vertebrate or an arthropod that transmits a pathogen from an infected host to a susceptible host. Bats and mosquitoes can be vectors. Vectors may be infected, such as the *Anopheles* mosquito that transmits *Plasmodium*. This means the pathogen reproduces inside the vector. Some vectors are carriers and are not said to be infected, because the pathogen does not reproduce inside the vector. A vector may also enable a pathogen to penetrate the outer defences of the potential host in a way that would not be possible unassisted. The varroa mite is a vector for several bee diseases. It can be infected or be a carrier.

Airborne droplets

Pathogens can be transmitted inside airborne droplets (aerosols) that are sneezed or coughed into the air and are suspended in air currents for a period of time before being inhaled or landing on a surface such as a table or tissue (a **fomite**). Transmission is indirect because it does not occur until later, when a susceptible host touches the fomite or inhales droplets, or it occurs over a longer distance. These tiny particles can travel considerable distances in air currents. Crowded indoor environments may promote the chances of airborne transmission, which explains the increase in respiratory infections during winter months.

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Soil, water, food and fomites

Non-living objects can carry a disease-causing agent from one host to another during the life cycle of a pathogen. Fomites are surfaces or objects that can carry an infectious agent. Examples include car tyres, clothing, eating utensils and mobile phones. Some diseases can also survive in soil and water until they find a suitable host. Crown gall and phytophthora dieback are two examples of diseases that can be spread by non-living objects.

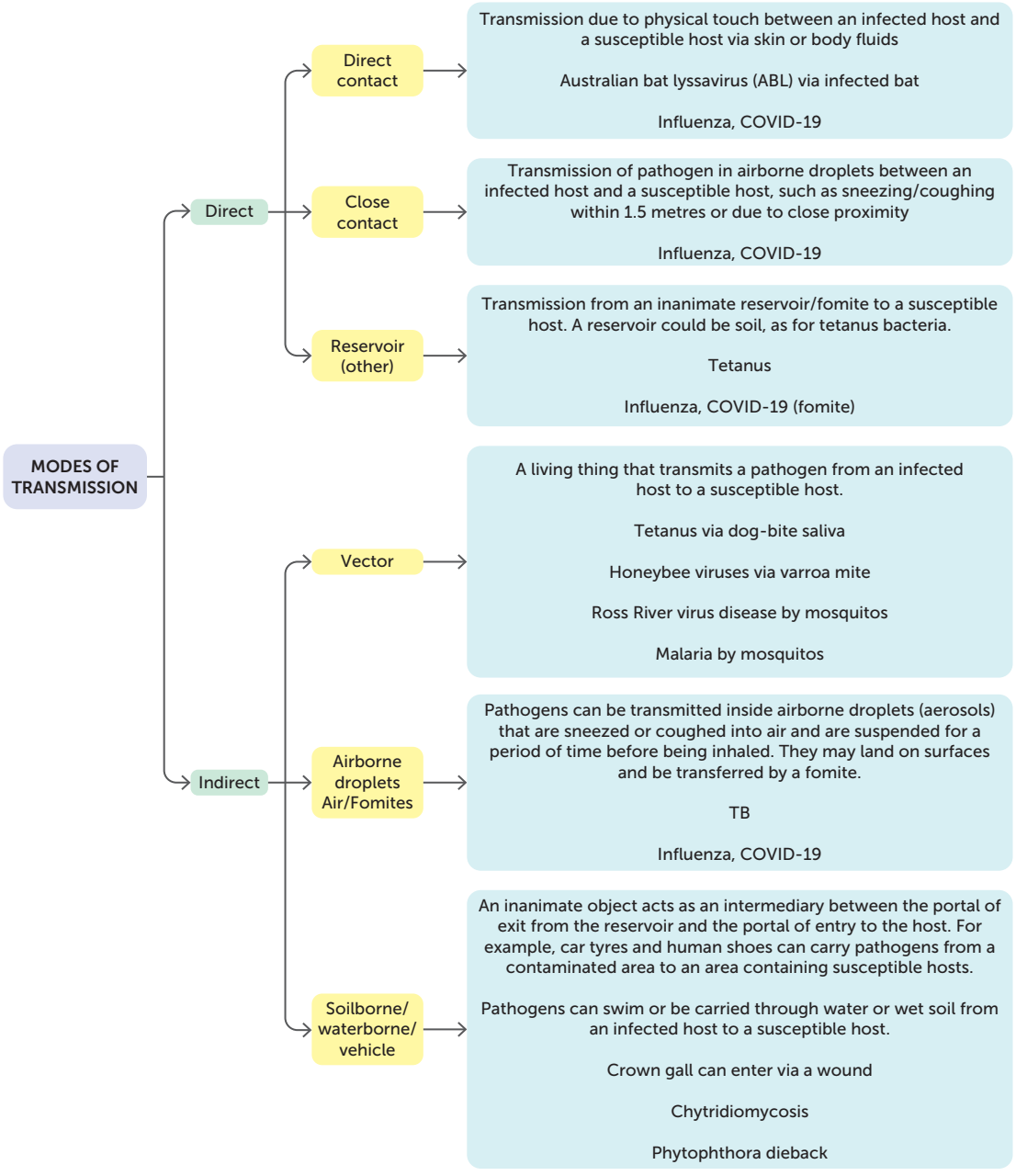

FIGURE 13.2 Examples of diseases and their modes of transmission

Key concept

Transmission of a pathogen can be direct or indirect. Direct transmission requires direct or close contact between an infected host or reservoir and a susceptible host. Indirect transmission is when vectors, fomites or airborne, soilborne or waterborne transmission transfers a pathogen from an infected host or reservoir to a susceptible host.

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Is food a vehicle for disease?

13.1 APPLICATION

The growing trend towards consuming take-away meals makes food poisoning an increasingly important problem in the developed world. Food should be eaten immediately after purchase or kept hot enough to kill bacteria. If it is to be stored, the food must be cooled very quickly to prevent the growth of bacteria that could cause food poisoning.

Questions

1. What steps do food manufacturers take to reduce the chances of food poisoning being caused by their food?
2. Use the weblink or other resources to find out the names of the pathogens that most commonly cause food poisoning from eating:
   a. poultry
   b. beef
   c. vegetables.

Foods that cause food poisoning
Read this resource to explore the different types of contaminated food.

Question set 13.2

REMEMBERING

1. Recall the names and descriptions of three direct modes of transmission.
2. Recall the names and descriptions of three indirect modes of transmission.
3. Define reservoir and describe three different types of reservoirs.

UNDERSTANDING

4. Explain the difference between direct and indirect transmission.
5. Explain the difference between direct transmission of airborne droplets and indirect transmission of airborne droplets.

13.3 PATHOGEN LIFE CYCLES FOR SOME SIGNIFICANT DISEASES

In this chapter, the life cycles for each of the following 10 diseases will be investigated:

- influenza, Ross River virus disease, viral diseases of honeybees, ABL (caused by viruses)
- TB, tetanus, crown gall of plants (caused by bacteria)
- chytridiomycosis (amphibian chytrid fungus disease) (caused by fungi)
- malaria, phytophthora dieback (jarrah dieback) (caused by a protist).

The pathogens are discussed in order from smallest to largest, based on the general relative size of the various types of pathogen: virus, bacteria, fungi, protist. Each life cycle requires a portal of entry, site for replication (sexual or asexual if non-viral), portal of exit, possible reservoir (other than human reservoir), and mode(s) of transmission.

Recall that viruses are not living and undergo replication instead of reproduction. In contrast, bacteria, fungi and protists undergo either sexual or asexual reproduction during their life cycles.

Life cycle of pathogenic viruses

Influenza

The infectious agents that cause influenza are the influenza A, B or C viruses. These single-stranded RNA viruses are usually transmitted from a respiratory tract, through the air inside airborne droplets, when an infected host coughs or sneezes. Viruses, found in the airborne droplets or spray, are inhaled and then enter the respiratory tract. Viruses will attach to epithelial cells along the respiratory tract if those cells contain suitable virus receptor cells. The virus will undergo viral replication inside the epithelial cells. Refer to Chapter 12 for the steps of viral replication.

After the release of new viruses from the epithelial cells, a cough or sneeze may send a spray of airborne droplets into the air. The droplets may be inhaled by someone in close proximity or (if small enough) become an aerosol and drift with air currents beyond the 1–2 metres, to be inhaled at a later time or land on a surface, making it a fomite. Therefore, transmission, although usually direct via close contact, can be indirect via airborne droplets or fomites.

Deaths caused by influenza or influenza-related illnesses were around 80 in WA during 2019, which is a significant increase compared with the number in the previous year. The symptoms of influenza can overlap with those of other illnesses common in winter. Symptoms that are more common with influenza and which can help distinguish the flu from ordinary colds and coughs include:

- **fever**
- severe fatigue
- general aches and pains
- cough
- vomiting and diarrhoea (in children).

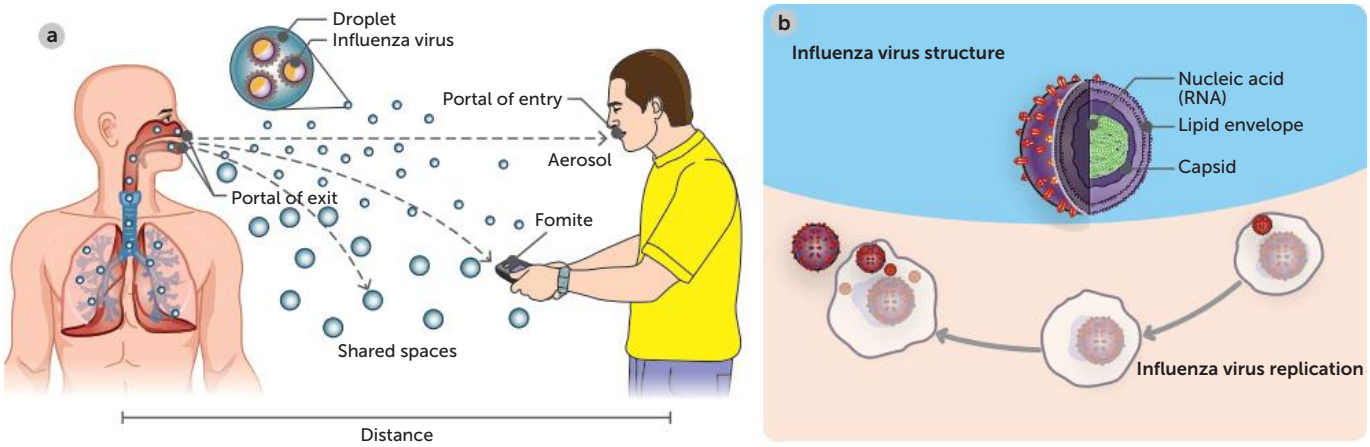

FIGURE 13.3 Influenza life cycle: **a** influenza virus portal of entry, modes of transmission, and portal of exit; **b** virus replicating inside epithelial cells in respiratory tract

Ross River virus disease (epidemic polyarthritis)

The infectious agent that causes Ross River virus disease is Ross River virus. The disease involves a rash and painful swollen joints, and it is sometimes misdiagnosed as arthritis (see Figure 12.7, page 412). Like the influenza virus, it is a single-stranded RNA virus. The virus is an alphavirus and is **endemic** in Australia, always present in some populations or regions. The virus is transmitted indirectly by female mosquito vectors, usually from marsupial reservoirs such as wallabies or kangaroos, to humans via blood feeds. **Outbreaks** in Australia have coincided with an increase in rainfall and flooding, which increases the potential breeding sites for the mosquito vectors. The disease is endemic in the south-west of WA, where outbreaks have been recorded, for example, in 2017. The **distribution** of the specific species of mosquitoes that can carry the virus largely determines where outbreaks occur. The major species of mosquitoes that can transmit the virus are *Aedes vigilax* and *A. camptorhynchus* near saltmarshes and coastal areas, and *Culex annulirostris* elsewhere.

Mosquitoes
Female mosquito blood feeds and the structure and function of the mosquito's proboscis

The portal of entry is via the skin and involves a female mosquito taking a blood feed. A blood feed is the method used by female mosquitoes to ingest blood. They insert their proboscis, a tube-like mouthpiece, into the skin and blood vessel of a host species to feed on blood. During the bite, the

mosquito's saliva is transferred to the potential host via one of the tubes in the proboscis, numbing the area and preventing the blood from clotting. Blood is sucked up from the potential host through another tube in the proboscis and into the gut of the mosquito. It's not until after the mosquito withdraws its proboscis that the site may become itchy. The saliva exiting the mosquito and the blood being ingested by the mosquito may both potentially carry pathogens. Female mosquitoes feed on humans when they are carrying eggs and need protein. Mosquitoes choose their targets through a combination of smell, heat and visual cues, and continue feeding until their abdomens are full. Female mosquitoes live for approximately 1 month and feed, on average, every two to three nights.

The main hosts are marsupials, but the mosquito transmits the virus from marsupials to humans. The virus does not usually transmit from human to human, and therefore the disease is infectious but not contagious. When a female mosquito vector takes a blood feed from a marsupial reservoir such as a western grey kangaroo, it pierces the skin and a blood vessel in order to suck blood. If the marsupial is a natural reservoir for the Ross River virus, the virus can be transmitted to the mosquito via the blood. If it is one of the correct species of mosquito, the virus finds receptors on mosquito epithelial cells to attach to in order to replicate. The viruses then move to the salivary glands, allowing for further transmission. The female mosquito vector may take another blood feed, this time from a susceptible human. As in the previous blood feed, the proboscis breaks the skin barrier and blood vessel wall. Saliva is injected into the site through one tube, and protein-containing blood is sucked up from the human host into the gut of the mosquito. In contrast to the previous blood feed, the virus is transmitted to the human host via the saliva. Again, the viruses will find receptors on cells to attach to. This time, however, they are muscle cells. After primary replication in the infected human skeletal muscle cells, the virus enters the blood and symptoms begin. It takes 3–9 days after transmission for symptoms of Ross River virus disease to appear. This period of time is known as the incubation period.

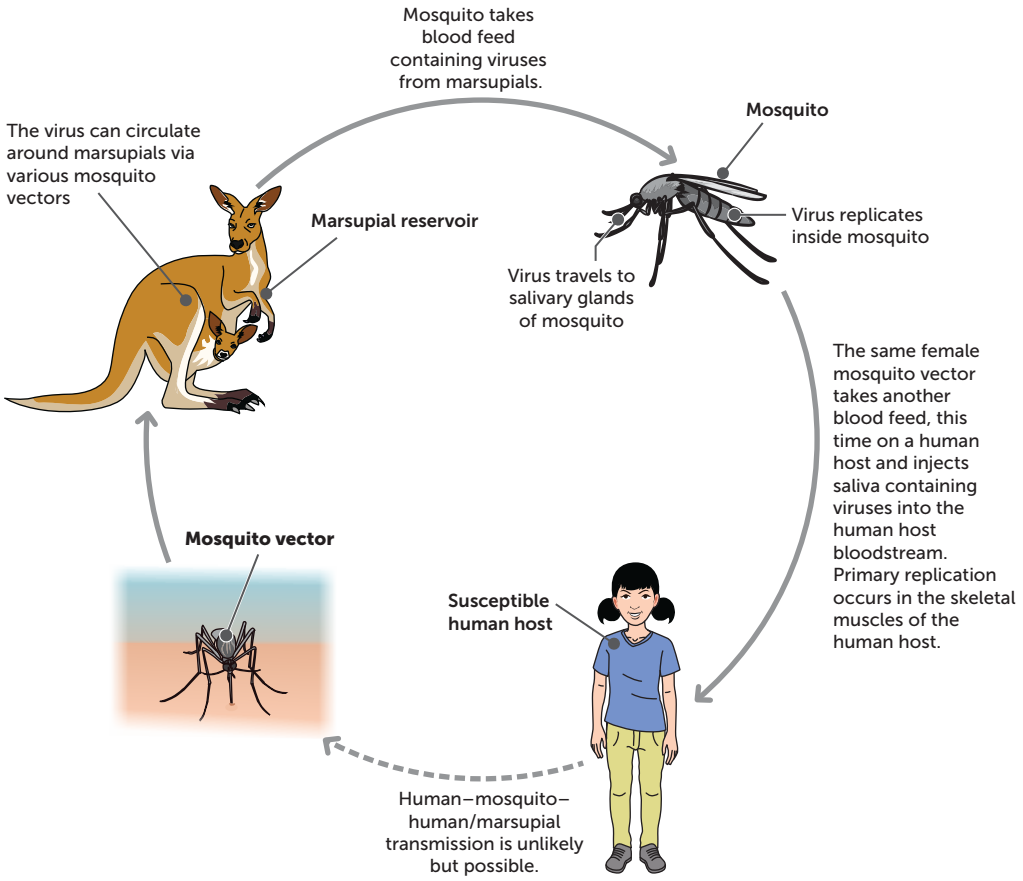

FIGURE 13.4 Life cycle of Ross River virus

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Viral diseases of honeybees

Honeybee viruses
Explore honeybee diseases further by reading this resource.

Sacbrood virus
Learn more about the honeybee virus found in WA: sacbrood virus.

There are many infectious agents that cause honeybee diseases, including 24 known honeybee viruses. Common diseases are sacbrood, black queen bee virus, chronic bee paralysis virus and deformed wing virus. Viruses may cause diseases that are asymptomatic, or they can be highly virulent and kill the hive. This significantly reduces the rate of pollination in the areas where the bees had previously resided, including the pollination of crops, which in turn affects human populations. Two of the common diseases found in honeybees are sacbrood and deformed wing virus disease.

Sacbrood disease

Sacbrood disease is caused by the sacbrood virus, which has infected bees internationally and was detected in WA in 1979. This disease mostly affects worker larvae, but can also infect adult honeybees. Infected larvae die just before pupation. The virus's mode of transmission may be foodborne: the virus can be carried by vector nurse bees when contaminated food is fed to the brood of larvae. The virus replicates inside the larval cells, causing the larvae to display unusual behaviour. The larvae turn onto their backs and lie stretched out with their heads lifted. After the larvae die, they turn light- to dark-brown, and an observer of the hive will see an unusual pattern of discoloured cappings. After the larvae dry out, they turn from brown to black and become brittle. Millions of viruses surround the dead larvae. When humans clean out dead larvae, they can cause the viruses to be disturbed and transported to other worker bees.

Rob Snyder/Bee Informed Partnership, University of Maryland (www.beeinformed.org) accessed May 2020.

FIGURE 13.5 Honeybee larvae affected by sacbrood virus

Deformed wing virus

Deformed wing virus (DWV) disease is caused by the deformed wing virus, which is usually transmitted indirectly by the varroa mite. The varroa mite is a significant pest in bee colonies all over the world. Fortunately, it has not successfully passed through the biosecurity in Australia.

Bee health
Read more about the impact of the varroa mite on honeybees by reading this resource.

One mode of transmission for DWV is thought to be via an attached parasitic varroa mite called *Varroa destructor*. The theory proposes that the parasitic mite feeds on bee blood and at the same time transmits the virus. However, the virus has been detected in bees during life stages not normally associated with the varroa mite, so other modes of transmission are likely in addition to transmission through the vector, such as vertical transmission from queen bee to offspring. The impact on honeybees can be asymptomatic or severe (in the form of deformed wings). Asymptomatic refers to the state of being infected but not experiencing any signs or symptoms.

Science Photo Library/Philippe Psaila

FIGURE 13.6 Newly emerged worker honeybee showing deformed wing virus (DWV) disease. A healthy winter bee can live for several months, but mites carrying DWV have compromised this bee's health, meaning it will probably not live more than a few days at most.

Australian bat lyssavirus

Australian bat lyssavirus causes the disease known as ABL. The virus was discovered in 1966. It is closely related to the rabies virus, but the rabies virus is not found in Australia. ABL infects Australian flying foxes (fruit bats) and microbats. The disease can be transmitted from bats to humans, to horses and to other bats. The disease affects the nervous system in humans, and can cause fatal **encephalitis** (inflammation of the brain).

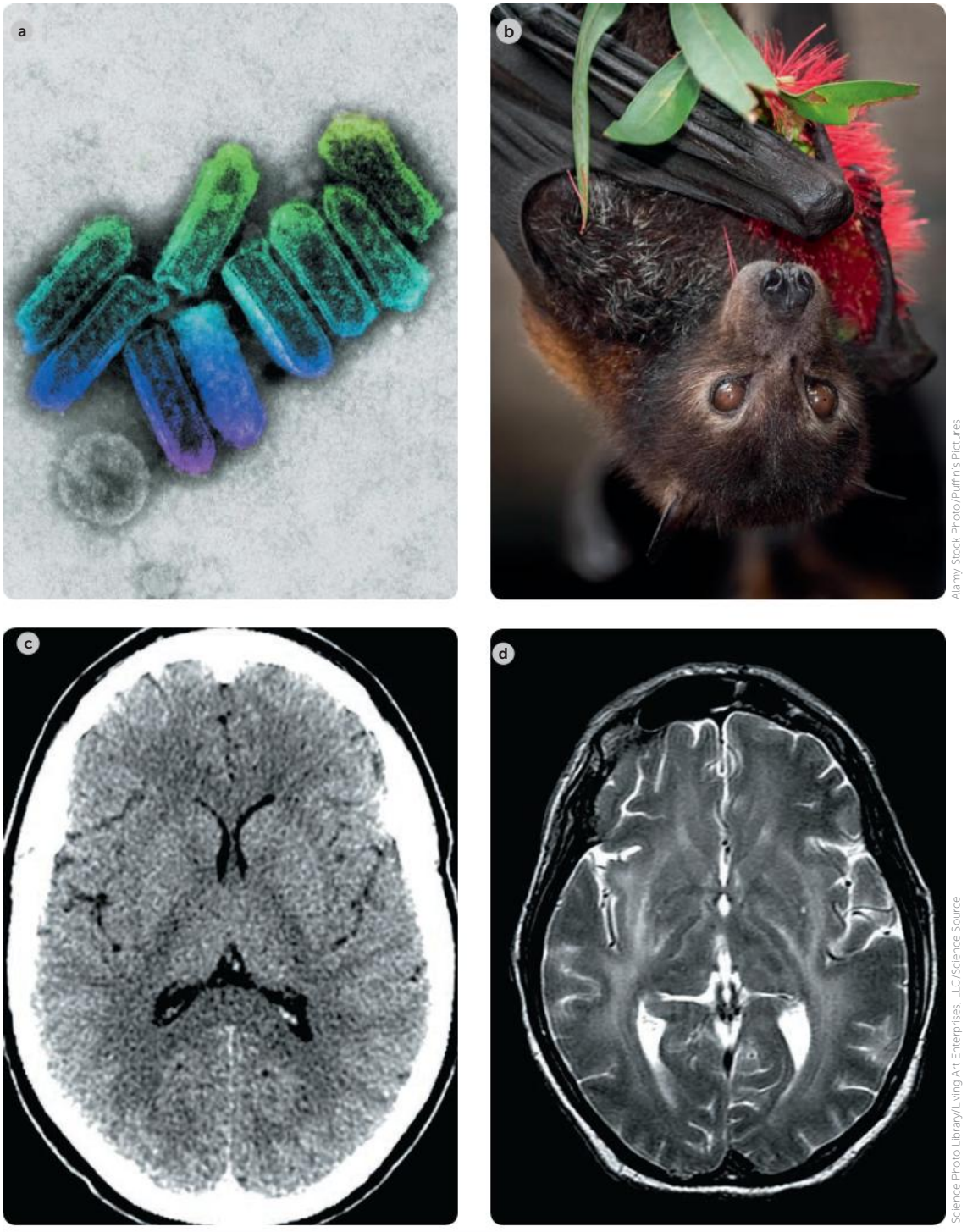

FIGURE 13.7 a A transmission electron micrograph showing colourised bullet-shaped ABL particles; **b** the bat host reservoir; **c** normal human brain; and **d** human brain with encephalitis

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Bats infected with the virus may show symptoms such as paralysis, weakness, tremors and seizures. The bats are a reservoir host for the disease. Some bats may be infected but asymptomatic, so all bats may be considered to be potential vectors. The virus multiplies within the bat and may travel to its salivary glands. The mode of transmission to humans or horses is direct contact with an infected bat host via a bite or scratch or other break in the skin, or via the mucous membranes. The virus replicates locally in the new host and then slowly travels through the sensory and motor nerves to the central nervous system (CNS), where it causes encephalitis. It then spreads to salivary glands and other organs via the peripheral nerves. Once the virus replicates within the salivary glands, the host becomes capable of infecting other animals or humans. The disease is not contagious between humans, but it is zoonotic.

The incubation period can be 10 days to over a year. This is followed by a course of symptoms in three phases. The first phase involves a slight fever, headache, nausea and sensitivity to light and wind. The person may feel sensations around the portal of entry, such as tingling, cold, itchiness, burning and pain. In most cases, the second phase of symptoms and signs, known as the excitatory phase, may be experienced as anxiety and unusual eye movements, and the eyes may become insensitive to touch. Facial muscles may weaken, and heart rate and respiratory rate may intensify, and these are followed by incontinence and constipation. This may precede the third phase, encephalitis, which leads to paralysis and coma. The prognosis (likely outcome) of the untreated disease is death. If promptly treated, however, the disease can sometimes be avoided. There have been no confirmed cases of ABL in WA, but there have been three cases of the disease elsewhere in Australia. All were fatal.

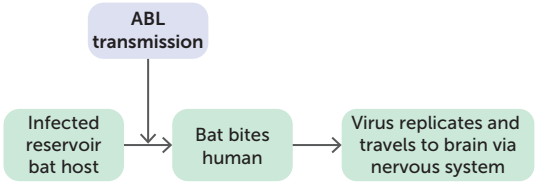

FIGURE 13.8 Life cycle of Australian bat lyssavirus

Life cycle of pathogenic bacteria

Tuberculosis

TB is caused by the infectious agent *Mycobacterium tuberculosis*. It is not common in Australia, but is very common in many developing countries. The **tubercle**-producing, rod-shaped bacterium mainly affects the lungs of the respiratory system. A tubercle is a small, round structure made of cells that is produced as a result of the infection. The disease spreads quickly when the density of the human host population is high and their immune system strength is low. In many socio-economically deprived areas, people often share limited space in housing and hospitals. Transmission is fast because susceptible hosts are always in close proximity. Much of the shared space is dusty, so both direct and indirect modes of transmission are possible.

When a person with TB coughs, tubercle bacilli are transported in fine droplets from the infected human host's lungs into the air, from where they can be inhaled by a susceptible person. Direct transmission occurs when a person is standing only 1–2 metres away from the infected person and inhales the airborne droplets. The TB bacteria rapidly move through that person's mouth, throat, trachea and bronchi, and deep into the lungs.

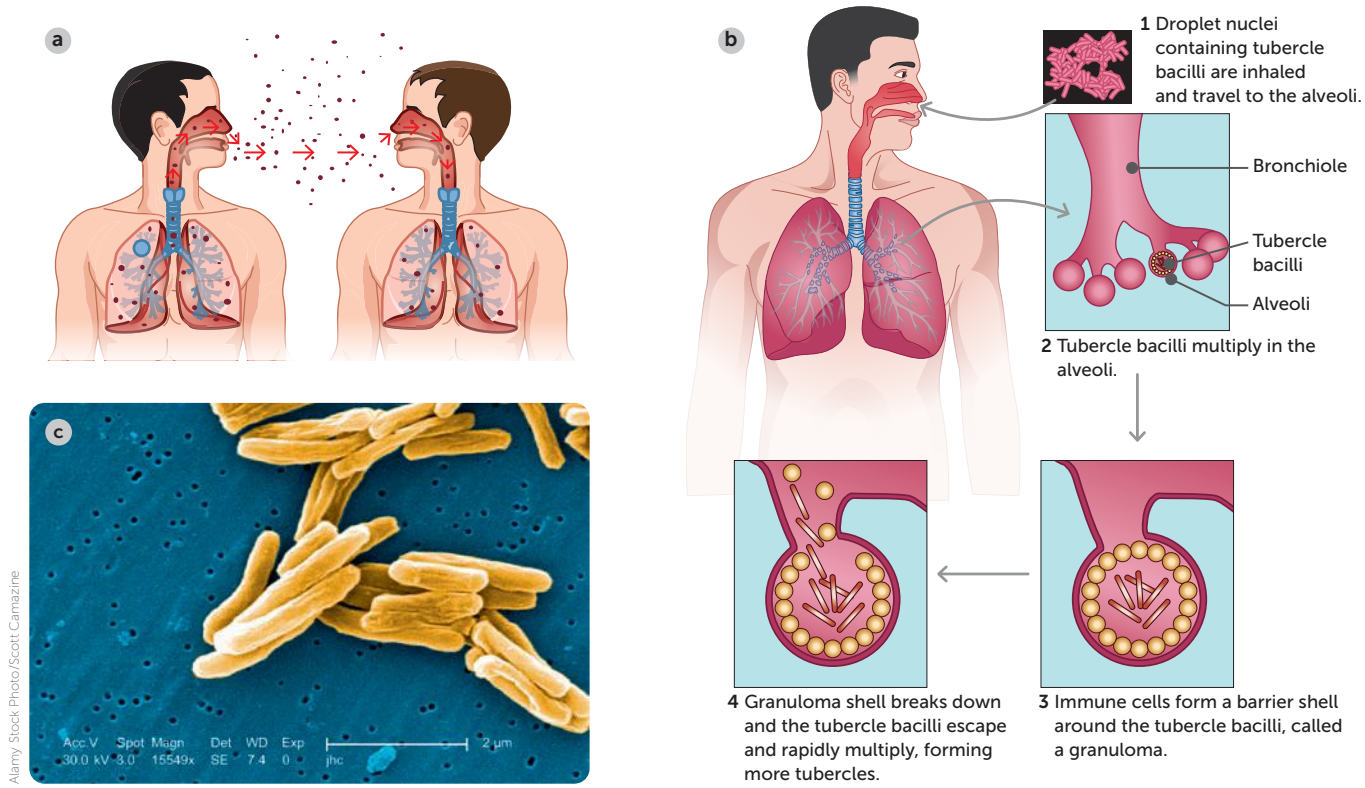

FIGURE 13.9 a Direct mode of transmission of TB via airborne droplets or particles; **b** the life cycle in and impact on the host; **c** *Mycobacterium tuberculosis*

Indirect transmission can also occur when an infected person coughs, sending droplets into the air, because the bacilli may land on a dusty surface. When the dust is disturbed and flies in the wind, it can carry the bacteria into the air and be inhaled.

When the bacteria enter the lungs, they are transported into the alveoli. In the alveoli, they multiply asexually by binary fission. Within 2–8 weeks, they are encountered by white blood cells (**phagocytes** such as **macrophages**). These are large cells that engulf invading pathogens by **phagocytosis**. The bacteria are able to evade the usual enzymes that destroy pathogens, because they survive inside macrophages, protected by the waxy mycolic acid in the bacterial cell walls. This strategy is known as a virulence factor. Instead of destroying the pathogen, the macrophage forms a barrier shell. It doesn't break the bacteria down, but does contain it and keep it under a degree of control. Eventually, the alveoli develop small, round lesions in the area of the infection, now named a tubercle. In this state, the host has latent TB. The bacteria remain dormant and symptoms are not experienced by the host. The host is not considered contagious, because they do not have the TB disease and the pathogen cannot spread to other people.

However, when macrophages can no longer contain the bacteria, the pathogens burst out and begin to rapidly multiply asexually, again. The infection has now become active, and the host is contagious and will experience symptoms. The pathogen may enter the bloodstream via capillaries and spread to other areas of the body, such as the lymph nodes or a bone.

The World Health Organization (WHO) recorded 1.5 million deaths due to tuberculosis in 2018. Tuberculosis deaths have been recorded for centuries. Nearly one-third of the world's population currently has latent TB. There are many factors that contribute to its persistence. One factor is that the main symptom is a cough, which is the main symptom of several other conditions. For TB, the cough has to be prolonged (2–3 weeks) to lead to testing. During that period, the person can continue to spread it, thinking they just have a cold or something minor.

SCIENTIFIC LITERACY

It's 'time to #EndTB'

It's 'time to #EndTB', says UN on World Tuberculosis Day.

A woman in Pakistan with TB went undiagnosed for 5 years because she could not afford the $2 transportation cost from her village to the closest hospital. TB is the world's deadliest infectious disease. WHO has announced that every day close to 30 000 people fall ill to TB, and that nearly 4500 people lose their lives to the disease. World Tuberculosis Day is on 24 March for the important reason of raising public awareness of the TB **pandemic**. It falls on the date when the bacterium that caused TB was first discovered. TB was first identified as a disease in 1882. It is preventable, treatable and curable. Why did 1.6 million people die from the disease in 2018, nearly 136 years after it was discovered?

Adapted from UN News, 'It's 'time to #EndTB' says UN on World Tuberculosis Day', March 2019

Questions

1. Discuss the following factors in relation to TB and how they have made it one of the world's most infectious disease killers. Use your knowledge from the chapter as well as the scientific literacy item.
   - **a** host factors
   - **b** pathogen factors
   - **c** modes of transmission
2. Determine the name of the doctor who discovered the TB bacterium. (Hint: it was the same doctor who developed a set of postulates to determine the specific cause of an infectious disease.)
3. Write out the doctor's postulates and how they would have been applied to finding out the specific cause of tuberculosis.

Tetanus

The disease tetanus is caused by the infectious agent *Clostridium tetani*. *C. tetani* is an obligate anaerobic Gram-positive bacillus. Entry into a susceptible host involves contact with the spore form of the bacterium, universally present in soil, typically via a deep wound. The spores are also found in animal intestines and faeces. The bacterial spores will not germinate in a superficial wound because they are **anaerobic bacteria** and will only germinate and multiply in hypoxic (low oxygen) conditions. Wounds that penetrate deeply into skin and soft tissues provide the hypoxic conditions required for germination of the spore and a portal of entry. After entry via a wound, the pathogen will start to multiply via binary fission and increase in numbers. During this process, toxins are released from the cells, transported through the bloodstream, and taken up and transported by nerve cells (neurons). The disease is caused not by the bacteria itself, but by the potent toxins that the bacteria produces during its growth. One major toxin is a neurotoxin called tetanospasmin, which affects the nervous system of its infected host. Once inside neurons, tetanus toxin cannot be neutralised by anti-toxin. The toxin blocks the release of inhibitory neurotransmitters (chemical messengers) in the central nervous system. This leaves excitatory nerve impulses unopposed, resulting in uncontrolled muscle spasms in skeletal muscles. Symptoms include lockjaw, which is the uncontrolled tightening of the jaw and the inability to open the mouth or swallow, and violent painful muscle spasms. The muscles of the body adopt an agonising posture known as opisthotonos: the extensor muscles of the back arch backwards and lock, and the arms flex to the chest with fists clenched. This can lead to other complications, such as loss of respiratory mechanisms, diaphragm dysfunction, airway obstruction and fractures associated with severe muscle spasm. The disease is infectious, but not contagious.

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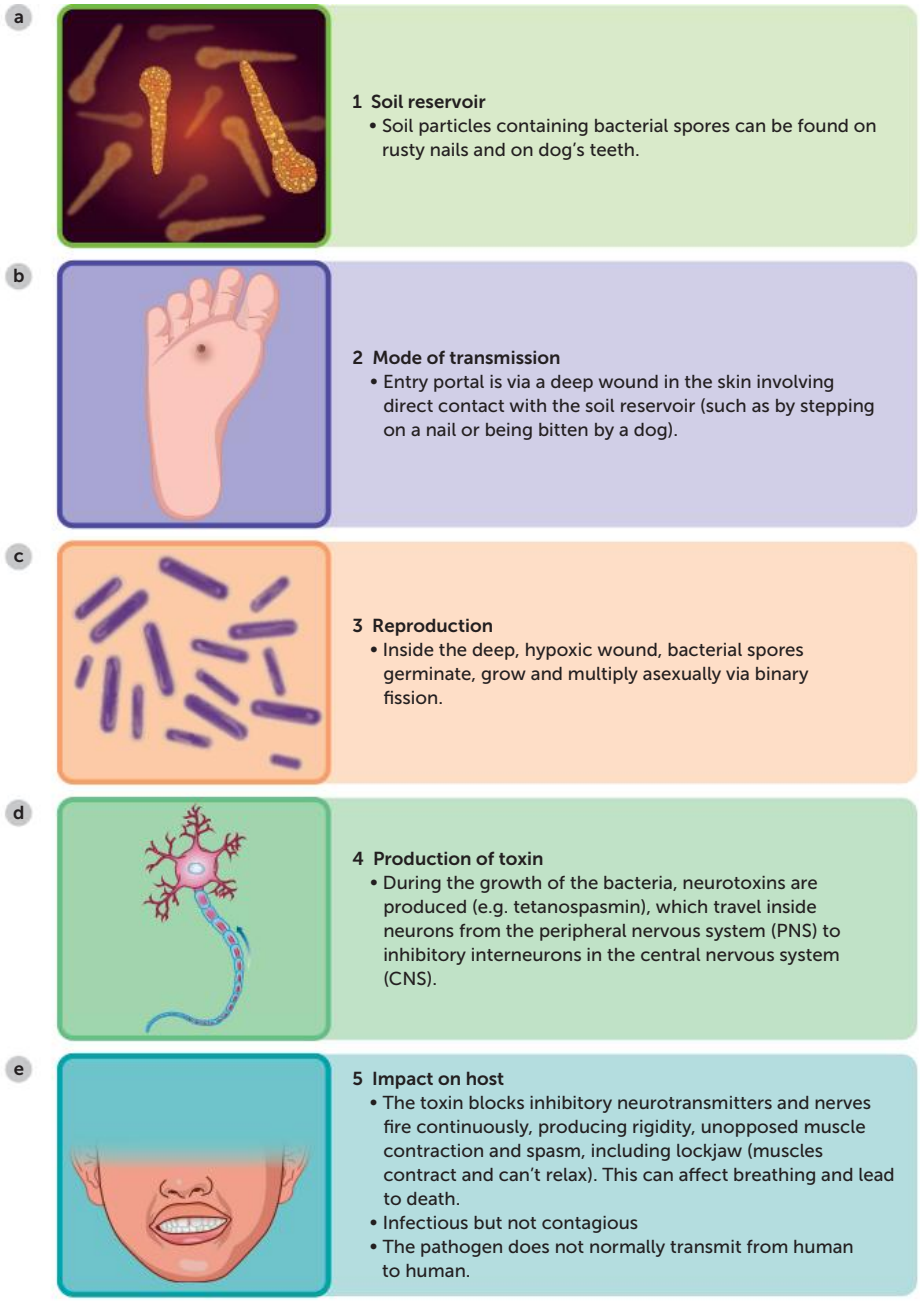

FIGURE 13.10 Life cycle of tetanus: **a** tetanus bacteria spores live in soil; **b** foot puncture wound; **c** inside the deep, hypoxic wound, bacterial spores germinate, grow and multiply asexually via binary fission; **d** toxins spread from the wound through the nerves and the blood to the central nervous system and the face; **e** 'lockjaw' (now called trismus) may occur, intense painful spasms of the jaw muscles

Crown gall

Crown gall disease of plants is caused by the infectious agent *Agrobacterium tumefaciens*. Bacteria can be transmitted from one host to another directly (via infected plant root to susceptible plant root contact) or indirectly [via grafting tools, car tyres, infested soil reservoirs, or movement of infested soil (such as by work boots or rain)]. Like the tetanus bacterium, *A. tumefaciens* requires a wound as a portal of entry. Unlike tetanus, the wound does not have to be deep.

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The disease can spread through susceptible crops such as stone fruits and roses. Farmers and gardeners have experienced financial loss from crown gall, particularly in nurseries where the plants are dug for sale. The disease can be found throughout Australia and has been a minor disease in roses and stone fruits in WA.

The bacterium that causes crown gall lives in the soil that surrounds the roots of plants. This bacterium can live in the soil as a decomposer for years without infecting a living host. When a plant is injured (either by mechanical transmission, insect feeding, or naturally) the damaged cells release compounds into the soil, attracting the bacterium to the wound site. Once inside the plant root, the bacterium replicates rapidly via binary fission, forming a tumour-like gall (a large cluster of undifferentiated cells that looks like a brown sphere) by integrating some of its DNA, which is contained in a circular plasmid, into the host's DNA. A plasmid is a small DNA molecule that is separate from the chromosomal DNA and can replicate on its own. Once the bacterial genomic material is incorporated into the host's genome, the normal plant cells are altered and genes for uncontrolled cell division are expressed. They multiply and form the gall structure.

Galls form on the **crown** of the plant and on the roots. The crown of a small plant is where the stem joins the roots. In trees, crowns are the sites where branches join the trunk. The galls can interrupt normal transport of water and nutrients. Consequently, a symptom that can be observed, in addition to the galls, is stunted growth. Galls can also form on the main stem above soil level, or on the branches. Old galls are dark and can grow to 30 centimetres. If the plant is suffering from moisture stress in addition to crown gall disease, then it is at risk of dying.

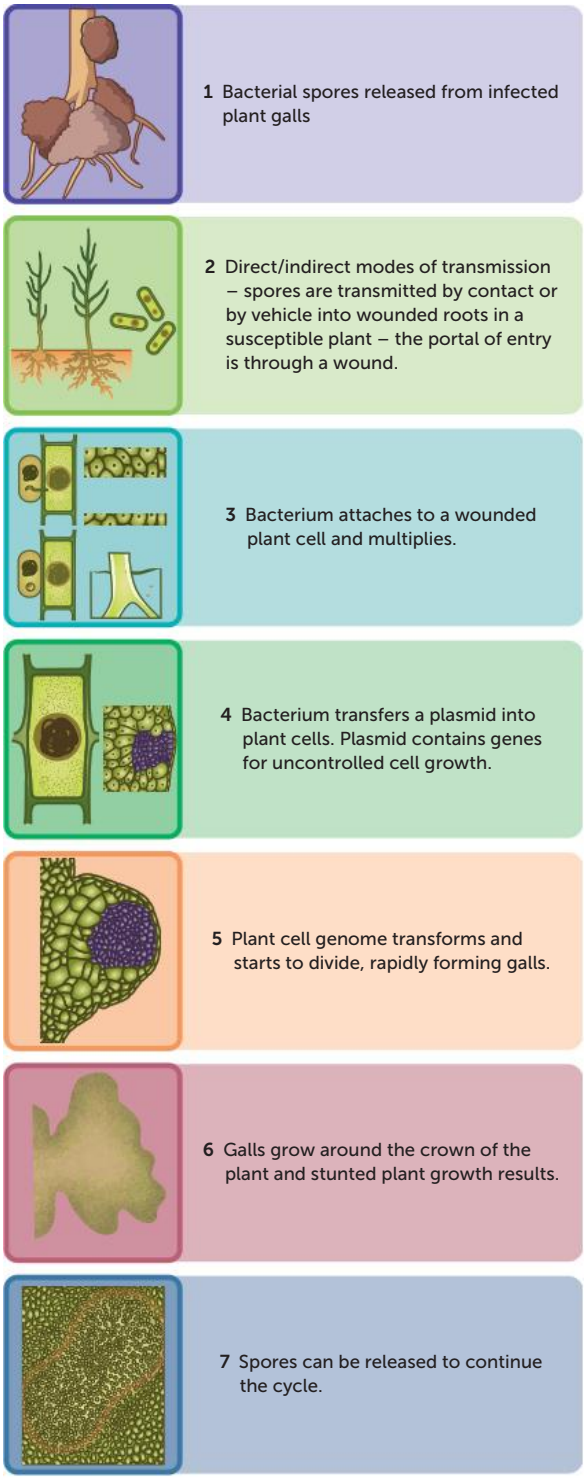

FIGURE 13.11 Life cycle of crown gall disease

Only the strains of bacteria that contain these tumour-inducing plasmids (Ti plasmid) can cause disease. Some strains of *A. tumefaciens* lack the specific plasmid and remain in the soil without causing disease. When the outside tumour cells shed into the soil, replicated bacteria can live in the soil and be carried off by water to infect neighbouring plants.

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Life cycle of pathogenic fungi

Chytridiomycosis

The disease chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis*. The chytrid fungus typically lives in water or soil and usually reproduces asexually only. Their **zoospores** have a single posterior **flagellum**, a whip-like tail, which provides the zoospore with locomotion (makes it motile and able to swim through the water).

The disease has been detected in all states of Australia and is affecting amphibians worldwide. In WA, the main area affected is the cooler south-west, possibly because the fungus is temperature sensitive. The disease causes amphibians to die, and at times mortality in a population is 100 per cent. The Global Amphibian Assessment of 2004 reported that one-third of the world's amphibian species are threatened.

Occasionally, transmission is direct via skin-to-skin contact; however, indirect transmission is more common. Indirect transmission is waterborne. Zoospores are released by infected frogs via a **zoosporangium (thallus)**, then swim through the water and attach to, and penetrate, the skin of a susceptible amphibian. Amphibians such as frogs are highly reliant on their skin for processes such as oxygen and carbon dioxide exchange (respiration), water absorption, pathogen defence and electrolyte transportation. Therefore, the impact on the frog is extensive. As the pathogen invades the skin, the outer **keratin** layers are damaged, disrupting respiration, salt regulation and osmoregulation (water balance). The skin thickens and hardens. As respiration, water and electrolytes decrease, so does the activity level of the frogs. Signs of the disease include lethargy, extension of the hind legs away from the body, and abnormal behaviour such as sitting in the sun instead of the shade.

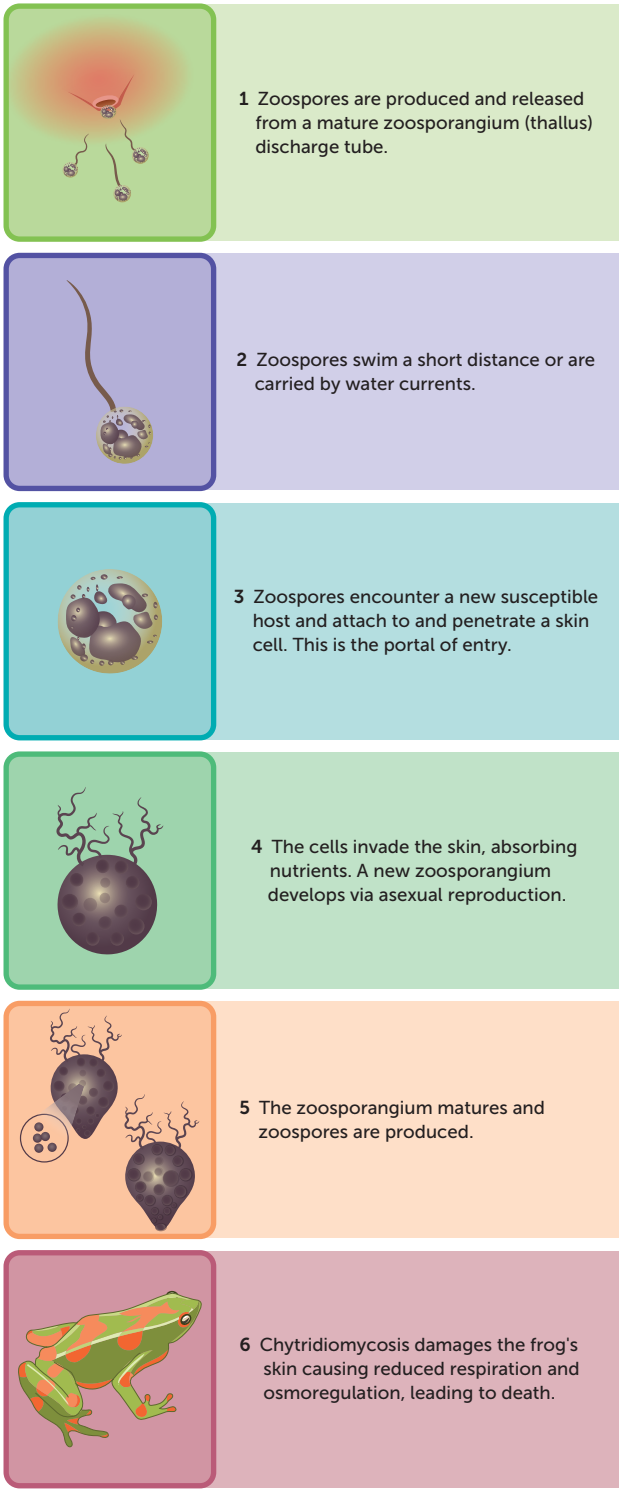

FIGURE 13.12 Life cycle of chytridiomycosis

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Note: chytrids lack hyphae, the typical filaments that are common in most fungal life cycles. Instead, they grow a roughly spherical, smooth-walled zoosporangium or thallus. Inside the thallus, asexual reproduction occurs, producing new zoospores. The thallus contains a plug that is removed once the thallus matures, releasing the zoospores into water.

The reasons why this fungus has spread so widely and so quickly could include (i) virulence of the pathogen (whether it has increased or evolved recently), (ii) the environment (whether there are more suitable environmental conditions for the growth and survival of the fungus in its reservoirs) and (iii) the host (whether there is reduced resistance to infection in frog populations). All these possibilities could potentially be caused by other factors, such as environmental changes or as yet undetected co-infections. Host, pathogen, environment and modes of transmission are interrelated.

Life cycle of pathogenic protists

Malaria

Malaria is caused by unicellular protists from the *Plasmodium* genus. *Plasmodium falciparum* is the most lethal, but there are six *Plasmodium* species that can cause malaria. *Plasmodium* species belong to a Protistan group resembling animal cells. The animal-type protists are referred to as protozoa. The infectious agents are transmitted indirectly to a host by the bite of a female *Anopheles* mosquito vector. The mosquito is the vector that completes the life cycle of the *Plasmodium* pathogen. The parasite requires both the mosquito and the human host. Sexual reproduction occurs in the female mosquito gut, which is why the mosquito is known as the definitive host. Asexual reproduction takes place in the liver and red blood cells of the human host, who is known as the intermediate host.

Australia was certified as malaria free in 1981 by WHO. However, hundreds of cases are recorded each year as people return from visiting other countries. Fortunately, the *Anopheles* mosquito vector does not occur naturally in Australia. In 2016, there were an estimated 216 million malaria cases in the world. Malaria kills one child every 2 minutes globally. WHO has a target of eliminating malaria by 2030. This goal is in serious danger of not being achieved, because the number of infected cases has surged in the last few years in some of Australia's poorer neighbours, such as Papua New Guinea.

When the mosquito penetrates the skin with its proboscis, it searches and pierces a blood vessel to access blood. Through one tube of the proboscis, saliva containing *Plasmodium* **sporozoites** (a relatively immature form of *Plasmodium*), is injected into the host, and blood is sucked up through a separate tube. The portal of entry is the same as the portal of exit, but one mosquito injects the pathogen in its saliva, and a different mosquito takes up the *Plasmodium* parasite within its blood feed. The injected sporozoite parasites travel through the bloodstream to the liver (Figure 13.13). After invading liver cells, the sporozoites divide repeatedly, by asexual reproduction, then develop and release thousands of **merozoites**. Merozoites are a relatively mature form of the *Plasmodium* pathogen. The merozoites leave the liver and enter the bloodstream, where they infect red blood cells within which they reproduce asexually again. The merozoites burst out and invade other red blood cells. Some of the merozoites break away from the red blood cell cycle and instead, form male and female **gametocytes** (underdeveloped male and female sex cells). The female *Anopheles* mosquito may bite an infected human, usually at dusk, ingesting the gametocytes as it takes the blood feed. Inside the mosquito gut, the gametocytes mature into male and female gametes and fuse to form zygotes, which is known as sexual reproduction. The zygotes penetrate and burrow through the wall of the mosquito stomach and form cysts. Sporozoites form within the cysts and migrate to the salivary glands of the mosquito, ready to infect a new host.

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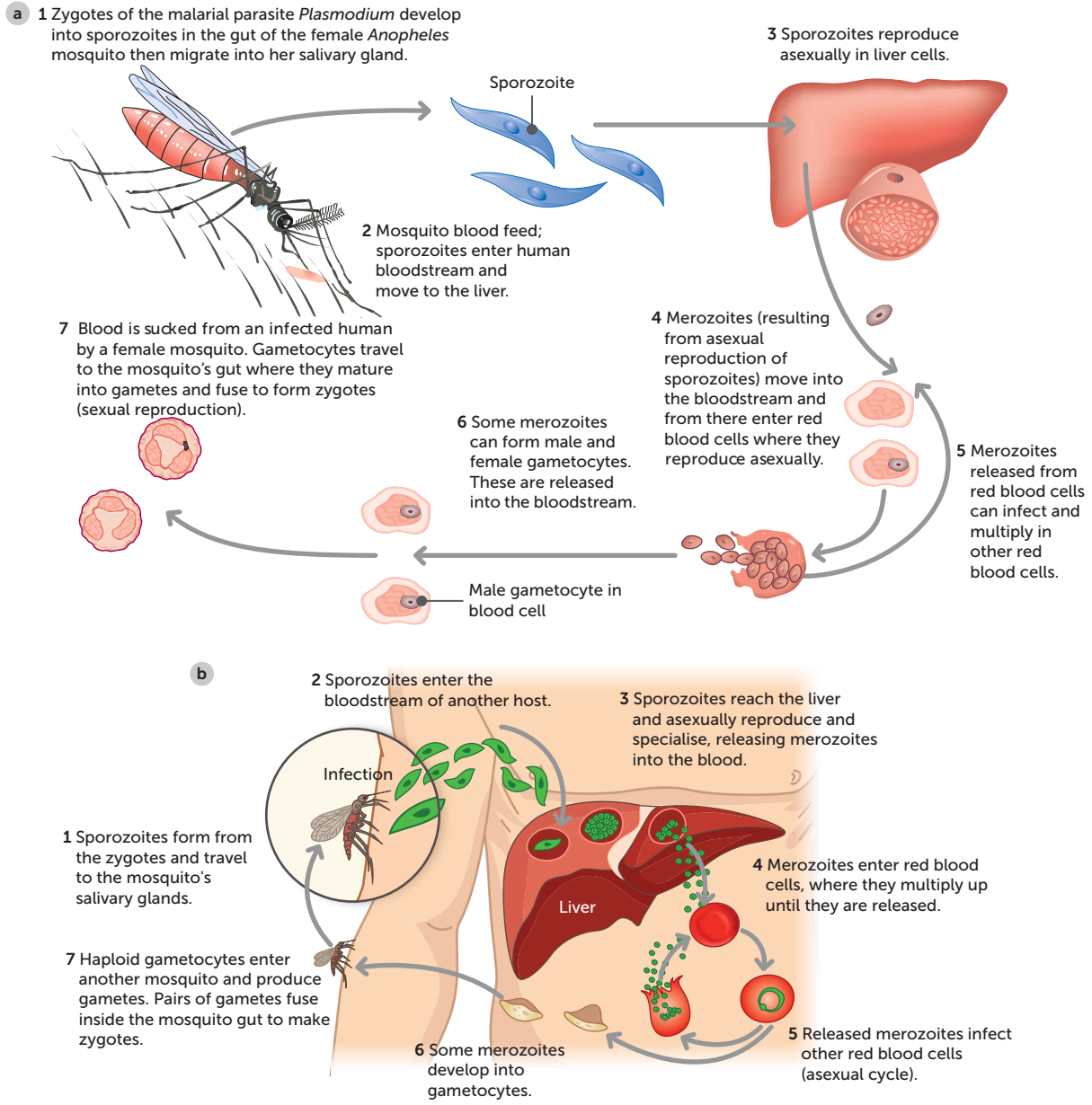

FIGURE 13.13 Life cycle of *Plasmodia* showing **a** its various forms and where they occur and **b** details of its asexual reproduction within the human liver and red blood cells

When infected red blood cells rupture in the process known as **lysis**, they release merozoites and their metabolic wastes into the bloodstream. This toxic release induces malarial headaches, chills and a burning fever. These symptoms eventually subside, but can recur when more cells are lysed, releasing more merozoites. Ongoing rupturing of red blood cells leads to anaemia (lack of red blood cells), which lowers the amount of oxygen that is transported to cells. If left untreated, the host may develop enlargement of the liver and spleen or, in the case of cerebral malaria, brain injury. This leads to death in severe cases.

Malaria life cycle
Watch the video, then draw your own life cycle of *Plasmodia*.

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Phytophthora dieback (jarrah dieback)

Phytophthora (pronounced fy-TOFF-thora) dieback is a disease caused by the water mould protist *Phytophthora cinnamomi*. It is a plant pathogen that affects plants in Australia such as jarrah, banksia and grass trees by attacking their root system. In WA, more than 40% of native plants are susceptible to the disease, particularly in the state's south-west.

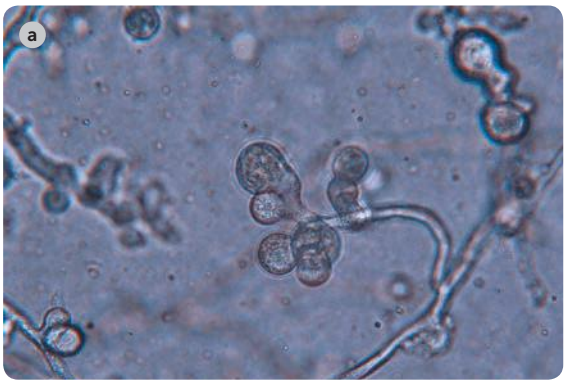

FIGURE 13.14 a *Phytophthora* hyphae; **b** injecting phosphite to manage the spread of *Phytophthora* in WA

Phytophthora dieback
Read more about the biology and ecology of Phytophthora dieback here.

Phytophthora reproduction
Zoospores are produced asexually inside sporangia; resilient chlamydospores are also produced asexually; zoospores are occasionally produced for reproduction in soil and plant tissue, but read about why reproduction is rare in Australia.

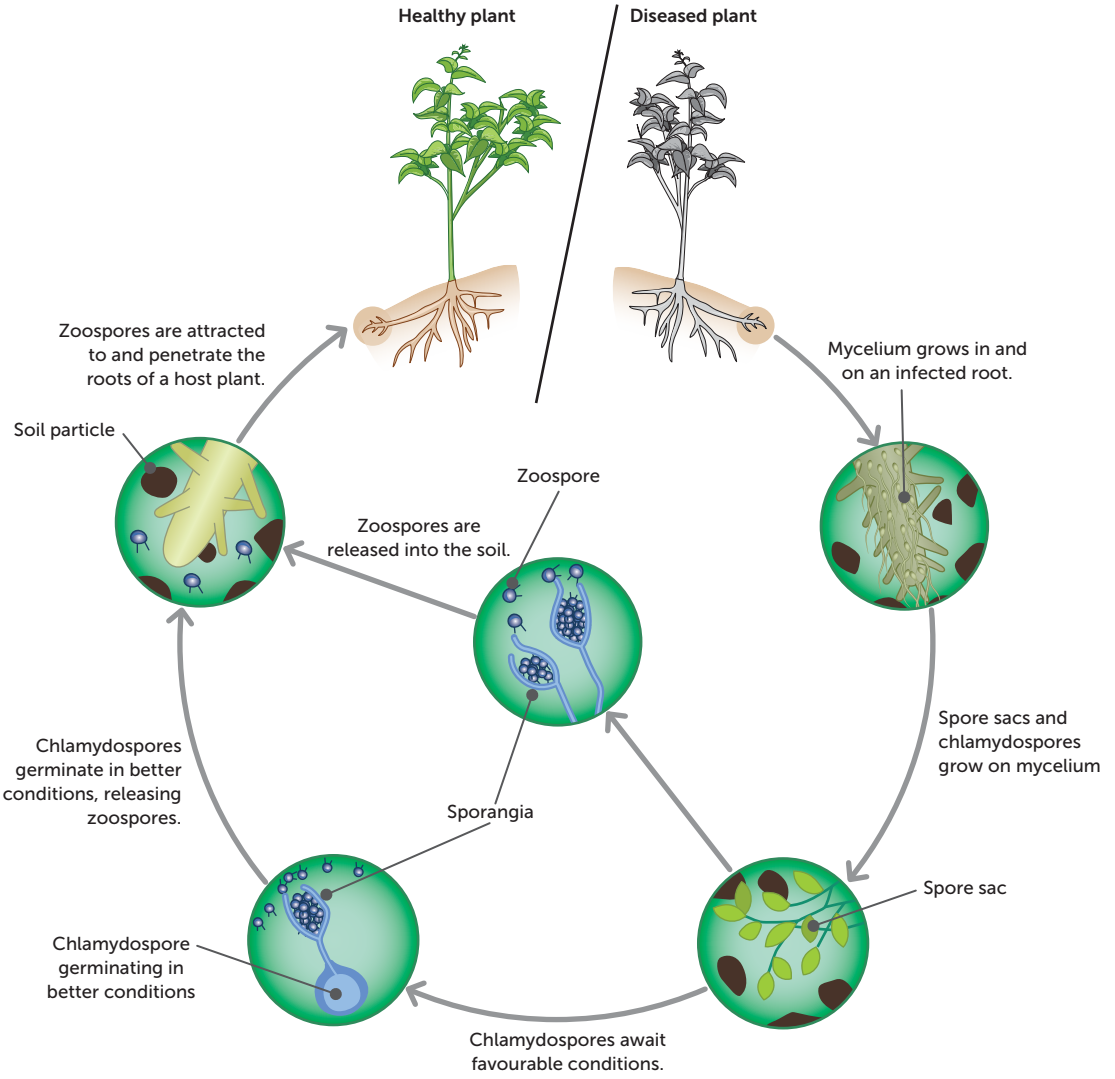

FIGURE 13.15 Life cycle of *Phytophthora*

Areas in WA that are currently experiencing spread of Phytophthora include Two People's Bay Nature Reserve and metropolitan Perth bushland areas such as Lightning Swamp Bushland. After infecting a plant such as a jarrah tree, it grows in the roots and rapidly kills it. It does not require a wound for a portal of entry, as does crown gall, although it can use such a portal. Like fungi, *Phytophthora cinnamomi* has threadlike hyphae and produces spores, but unlike fungi its cell walls are cellulose-based rather than chitin-based.

The usual portal of entry is via root tips. Zoospores swim through the soil water and attach to the root tip cells of susceptible host plants. After attachment, the zoospores grow long, thin microscopic filaments of cells. Bundles of a few filaments form the **mycelial threads** known as hyphae that grow on the surface of infected roots, absorbing nutrients. They then produce sporangia, which release zoospores. Once the zoospores have swum to and infected a plant, they produce long-lived chlamydospores (which can survive unfavourable conditions), sexual oospores and further sporangia.

When the soil is dry, *Phytophthora cinnamomi* can remain dormant in the chlamydospore form for long periods, until it rains again and conditions are favourable.

The mode of transmission is usually indirect via vehicles such as cars and shoes carrying contaminated soil. The disease is soilborne and can spread faster when it rains. Transmission can be direct from infected to susceptible plants by root-to-root contact. Impact on the plant is swift after transmission. *P. cinnamomi* attacks the roots, causing root rot. The growth of the protist reduces the ability of the plant to absorb water and nutrients. Dieback is progressive and death may follow.

Phytophthora dieback: why is the disease such a massive problem in Western Australia?

CASE STUDY

Root rot is a term describing one of the impacts *Phytophthora cinnamomi* has on plants. It affects over 200 threatened native plants and is considered a highly problematic invasive species. The Australian Government developed a threat abatement plan for the disease in 2017. The Department of Parks and Wildlife in WA has stated that the disease affects more than 40% of the native plant species and half of the endangered ones in the south-west of WA. Biodiversity in WA is declining because of this disease.

The pathogen is able to be transmitted via zoospores that are attracted to roots

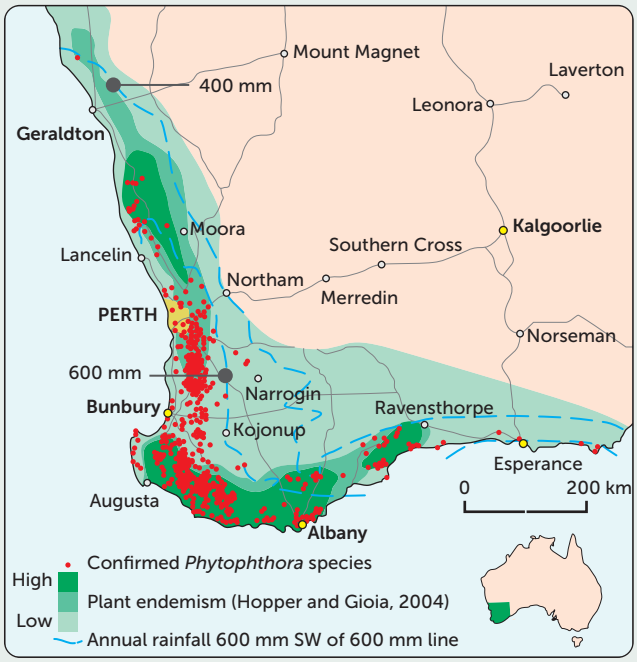

FIGURE 13.16 Map showing the association between *Phytophthora cinnamomi* distribution and higher rainfall areas with endemic (native) plants

in the soil, and they actively swim towards susceptible root tips through wet soil from a infected site. They do not require a wound. The entry portal is the finer tips of roots in plants. The pathogen germinates and grows a cyst before growing thread-like structures known as hyphae. In the hyphae form, the protist can grow into the root cells to absorb nutrients.

Phytophthora cinnamomi's life cycle is not limited to one mode of transmission. The pathogen can survive in dry, unfavourable soil conditions as chlamydospores until conditions become favourable (warm and moist). Chlamydospores may undergo sexual reproduction or germinate to form sporangia to propagate more zoospores. Other indirect modes of transmission are vehicles, such as contaminated grafting tools, car tyres and human footwear, all of which can transport contaminated soil to new places. In addition to indirect transmission, the disease can spread via direct root-to-root contact between infected and susceptible plants.

There is no known cure for the disease phytophthora dieback.

Questions

1 How is *Phytophthora cinnamomi* transmitted from host to host?
2 How does *Phytophthora cinnamomi* reproduce?
3 Why are outbreaks of *Phytophthora cinnamomi* so difficult to contain and control?

Question set 13.3

REMEMBERING

1 Copy and complete this summary table for the 10 diseases covered in this section and their life cycles.

DISEASE	PATHOGEN TYPE	PATHOGEN NAME	MODE/S OF TRANSMISSION (most common to least common)	LIFE CYCLE SPECIFICATIONS [portal of entry, site(s) of replication (sexual/asexual reproduction), reservoir(s), portal of exit]

UNDERSTANDING

2 Explain why ABL's rate of spread may be lower or higher than the other kinds of viral diseases mentioned (influenza, viral diseases of the honey bee and Ross River virus disease).
3 Draw a life cycle summary for a chosen bacterial disease.
4 Draw the life cycle for chytridiomycosis.
5 Draw a life cycle for one of the protistan diseases.

13.4 FACTORS THAT AFFECT THE SPREAD OF A DISEASE

Control of any disease is related to **thresholds**. A threshold is a certain magnitude that must be exceeded before a result can be produced. Certain parameters or host/pathogen/vector population sizes must be exceeded for a specific infectious disease to spread. For example, a disease may be unable to spread if the population is below a critical size. Since 1967, at least 39 new pathogens have been identified, including the human immunodeficiency virus (HIV), Ebola virus, SARS-CoV-1 and SARS-CoV-2. Other centuries-old threats, such as pandemic influenza, malaria and tuberculosis, continue to pose a threat to health through a combination of mutation, rising resistance to

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antimicrobial medicines, **globalisation**, **urbanisation**, **climate change** and poor health systems. Climate-sensitive vector-borne diseases are likely to be emerging due to climate changes and environmental changes, such as increased irrigation.

Spread is the transmission of a pathogen to susceptible hosts over a wider area and into new populations. The rate of spread of a specific disease is affected by a range of **interrelated factors** (factors that depend on and have an effect on one another), including growth of the pathogen population, density of the host population and modes of transmission. All play a vital part in spread and all three factors need to reach particular thresholds or spread will be limited. Successful replication in host cells, high-density living, and airborne droplet and fomite transmission have meant that the rate of spread of COVID-19 has been relatively fast.

Growth of the pathogen population

An increase in the growth of a pathogen population can lead to an increase in the spread of a disease. Greater abundance of a pathogen means the risk of transmission is higher. Some pathogens, such as viruses, have a very high rate of replication, which increases their chance of spread. However, spread relies on all three interrelated factors, so if there are limited hosts or reservoirs, then the pathogen will not survive. A reservoir is where a pathogen resides and survives. Some pathogens reside and survive in a host, but other pathogens require a vector or reservoir other than the host they infect. Scientists are monitoring the increase in Australian bat lyssavirus because the pathogen causes a fatal disease in humans. The virus was first identified in 1996 and has been found in four kinds of flying foxes (fruit bats) and one species of insect-eating microbat. It is spread to humans by the saliva of infected bats when the saliva comes into contact with mucous membranes or broken skin, or through bat bites or scratches. Infection is fatal, but the infection rate in humans is low. One reason for this is, unlike mosquito vectors, bats do not use humans as a primary source of food. They consume insects or fruits. In addition, the mode of transmission is direct contact. Humans usually only come into contact with a flying fox when one is injured. Therefore, the mode of transmission is a limiting factor for this pathogen. Regardless of the growth of the pathogen population, without a more effective mode of transmission, the spread of ABL in humans is limited. However, some scientists are concerned that this zoonotic disease will emerge as an **epidemic** due to rapid environmental change and the way that is affecting bats.

Density of the host population

An increase in the density of a population can lead to an increase in the spread of a disease. The human population has increased to more than seven billion. This increase, coupled with urbanisation, has led to much higher density living. Urbanisation is the movement of people from rural areas to towns and cities. A higher **population density** means more people are in a particular area at the same time. This means that for any given pathogen present in the community, more infected individuals will come into contact with susceptible individuals. This is a significant problem in relation to pathogens that transmit via direct contact. Many animal vector populations are affected by habitat loss. The animal vectors themselves may be living in higher density populations due to limited habitat space, and humans are encroaching on what was natural habitat for living space and agriculture.

However, spread is dependent not just on the density of the host population, but also on the pathogen population growth. If there are limited pathogens, then spread will not increase. The mode of transmission can also affect spread, and an increase in either the pathogen population or the host density will have negligible impact on the spread of a pathogen if it cannot be transmitted. For density-dependent pathogens, there is theoretically a level of density below which transmission is inefficient and the pathogen cannot persist. For density-independent pathogens, transmission may be relatively unaffected by host density and relatively more affected by vector density. The spread of TB was discussed earlier in this chapter. The mode of transmission of TB can be direct, by close proximity via airborne droplets containing the pathogen. Therefore, in areas of the world where there is urbanisation and high-density living, the rate of spread is expected to be high. It is particularly high in

areas that contain high levels of the pathogen population and low level of healthcare, which together allow the transmission to be very effective. In poor countries, TB spreads quickly and easily because all three interrelated factors exceed their threshold. In higher socio-economic areas of the world, healthcare is more accessible, disenabling the modes of transmission and decreasing the pathogen population through medication and prevention measures. A high density of the host population by itself is not the sole determiner of the spread of an infectious disease.

Mode of transmission

Pathogens have one or more unique transmission modes specific to them. Some infectious agents are easily transmitted (that is, they are very contagious), whereas others are not easily transmitted. Some of those that are easily transmitted are not very likely to cause disease (that is, they are not very virulent). The most worrisome infectious agents are those that are both very contagious and very virulent.

Some pathogens have multiple modes of transmission. One mode of transmission can switch to another depending on the environmental conditions. Phytophthora is an example of a disease that can follow two different pathways depending on the environmental conditions. When conditions are ideal, zoospores are produced that may swim to another susceptible plant. When conditions are not ideal, for example during dry periods, resilient chlamydospores may be produced that can survive for long periods of time. When conditions improve, zoospores develop and complete the transmission.

Pathogens may be transmitted through direct and/or indirect contact. When pathogens can be transmitted via several modes of transmission, this increases their chance of spreading. However, the pathogen still relies on a threshold number of pathogens being present and a certain level of host population density to successfully spread. If there are multiple modes of transmission but few hosts or pathogens, then there is a limiting factor and spread will be limited.

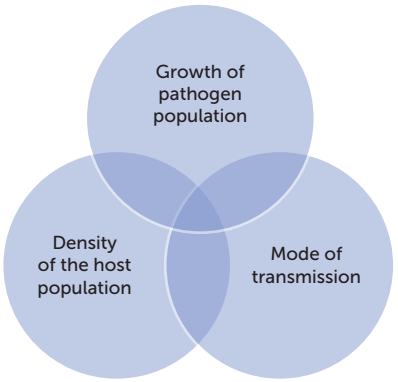

FIGURE 13.17 Interrelated factors influencing the spread of pathogens

Globalisation

For most of human history, continental populations have been relatively isolated from one another. Only relatively recently has there been extensive contact between the humans, plants and animals of different continents. Initially, new infectious diseases could spread only as fast and far as people could walk. Then they started spreading as fast and far as horses could gallop and ships could sail. Globalisation is a modern phenomenon and it is having a big impact on the spread of disease. Globalisation is the process by which the world is becoming increasingly interconnected as a result of massively increased trade, economic, travel and cultural exchange. The efficiency, speed and reach of modern transport puts people at risk from the emergence of new strains of familiar diseases, and from completely new diseases. In addition, the growth of global economic activity, tourism and human migration is leading to ever more cases of the movement of both disease vectors and the diseases they carry.

Disease can quickly spread to new areas if infected hosts travel into different regions or areas of the globe. Tourism and trade such as in poultry and fruit can facilitate the transmission and spread of diseases. Transmission involves the transport of a pathogen from reservoir to susceptible host, and spread involves transmission into wider areas and new populations. Transmission and spread of disease can be due to war, refugees seeking safety, and legal and illegal trade in wildlife. Pathogens can be transported in the traveller or on objects being transported. In our increasingly interconnected world, new diseases are emerging at an unprecedented rate, and they have the ability to cross borders and spread rapidly, as has been the case with COVID-19.

Measles is a highly contagious viral disease spread by tiny airborne droplets when infected people sneeze or cough. It had virtually been eliminated from WA for approximately 20 years by vaccination and isolation of cases. Outbreaks, however, have occurred when an infected international tourist has visited the state or via a returning WA traveller who has been infected. The incubation period is 7–21 days. Therefore, someone can be unaware they are contagious. Australians currently rely on herd immunity to keep the minority of unvaccinated, vulnerable individuals protected and to prevent spread. A fly-in, fly-out worker infected with the virus was diagnosed on the 12 October 2019, 10 days after flying into Christmas Creek mine site in the Pilbara. The worker was then promptly quarantined. However, in addition to the workers at the mine site, workers at the airport and people on the plane had been exposed to the infection.

African swine fever (ASF) virus is endemic to sub-Saharan Africa. A disease is said to be endemic when it is always present in the region. ASF is caused by a DNA-type virus, and there is no vaccine or cure. It can spread directly through live or dead pigs and their body fluids. It can also be spread indirectly through animal feed that contains pork infected with ASF, or via fomites such as transport storage surfaces, shoes and clothes. The virus has high environmental resistance; that is, it can survive in extreme, dry temperatures.

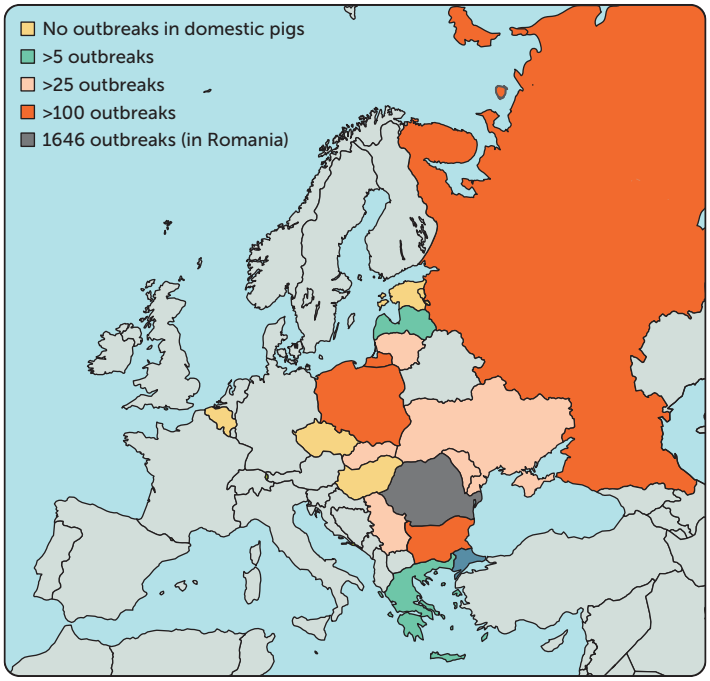

FIGURE 13.18 Number of outbreaks of African swine fever, September 2019 – March 2020

The recent spread of ASF has been the biggest animal disease outbreak in history. It has killed more than a quarter of the world's pigs. This has occurred due to the movement of pigs and pig products across borders, exacerbated by the fact that farmers in South-East Asia were hesitant to report the disease for fear of stocks being culled and loss of livelihood, with no offers of compensation being given to them. The outbreak was first reported in north-eastern China in August 2018, following which the highly contagious, often fatal pig disease quickly swept through


Getty Images/MANAN VATSYAYANA

FIGURE 13.19 Processing a pig killed by African swine fever

the country, causing the death or culling of more than 1 million pigs. The epidemic then expanded across borders to become a pandemic. An epidemic is a sudden increase in the prevalence of a particular disease that spreads rapidly through a region or nation after an outbreak. A pandemic is the rapid spread of a particular disease throughout the world, crossing international borders. By October 2019, ASF had jumped borders to Vietnam, Cambodia, Mongolia, Hong Kong, North Korea, South Korea, Lao, Myanmar, The Philippines, Timor-Leste and parts of Europe.

Mosquito-borne diseases and climate change

The distributions of mosquito-borne diseases, such as malaria and Ross River virus disease, are being affected by global climatic changes. Distribution refers to the area of occurrence of an infectious disease, including the patterns of spread in geographical areas. The distribution of a disease may not be consistent or uniform, but instead concentrated in particular areas. Global climate change, as we are experiencing, encompasses increases in global average air and ocean

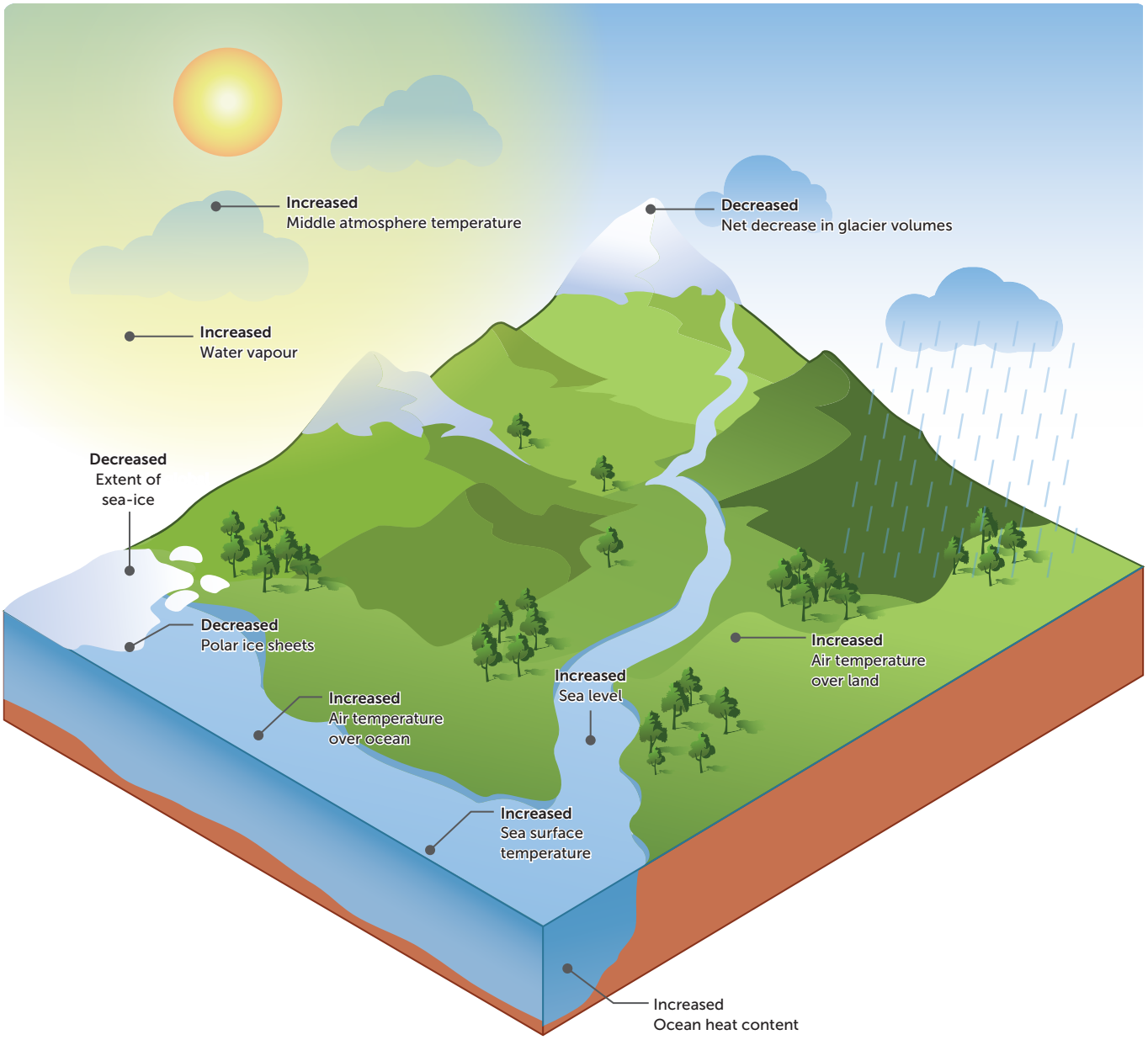

FIGURE 13.20 Changes in the global climate system

temperatures, rising global sea levels, long-term sustained widespread reduction of snow and ice cover, and changes in atmospheric and ocean circulation and regional weather patterns, which influence seasonal rainfall conditions (Figure 13.20).

Climate change has led to an increase in more severe weather events, including heat waves and storms, and these are expected to become more common. The causes of climate change are complex, but one root cause is the following series of events. The gases released during the burning of fossil fuels and agriculture are predominantly greenhouse gases, such as carbon dioxide, methane and water vapour. These atmospheric gases trap solar energy that otherwise would radiate out of the atmosphere, retaining heat in our atmosphere. Increased levels of these gases in our atmosphere has resulted in a rise in average global temperatures. The higher temperatures are causing the climate to change. This affects the weather. Some geographic areas will have more rainfall, and some will have more drought.

Projecting future climate change
Read the information, watch the video and describe the link between greenhouse gases and global warming. Describe how this has affected Australia.

13.2 APPLICATION

Models have been used to project future climate change, but scientists cannot know for certain the quantities and sources of greenhouse gas emissions. Apply your knowledge of the scientific method (such as observation, hypotheses and variables) to describe how scientists use past knowledge to project future changes.

The distribution of mosquito-borne diseases is likely to increase as a result of climate change, because warmer temperatures and extra water bodies will increase the activity of mosquitoes and the number of breeding grounds where mosquitoes can reproduce. An increase in the incidence of the mosquito vector leads to an increase in transmission.

For example, malaria is transferred by the female *Anopheles* mosquito vector. Mosquitoes will carry malaria with them when they spread to new areas, increasing the distribution of the pathogen, which may increase transmission rates. In addition, malarial activity varies seasonally in the areas in which it is endemic. The link between malaria and extreme climatic events has long been studied. In India early last century, for example, the river-irrigated Punjab region experienced periodic malaria epidemics. Excessive monsoon rainfall and high humidity were identified early on as a major influence, because they enhanced the breeding and survival of mosquitoes.

Antibiotic resistance

Evolution is the cumulative, gradual, inheritable change in a population over generations and usually a very long time. The pathogens investigated in this text are all microscopic, but bacteria and viruses stand out as being the smallest. Small and unicellular, bacteria have one enormous advantage over larger, multicellular organisms, and that is their reproductive rate. Bacteria can reproduce asexually within minutes by binary fission. If a mutation or alteration causes a tiny change that gives the bacterium just a tiny advantage, then it can spread rapidly due to the high reproductive rate. Environmental factors impose a selective force on populations, including pathogen populations. The environment is changing at a faster rate than at earlier times in history. Factors such as average air temperature and habitat loss are driving forces behind a more rapid evolution of pathogens.

Antibiotics are medications that destroy or slow down the growth of bacteria. They are also known as antibacterials because they only act on bacteria. Antibiotics have been misused over time, and this has led to the evolution of bacteria with resistance to antibiotics. When antibiotics were first synthesised, they killed most bacteria and were used to treat many affected people and pets. All bacteria in a population died, including any resistant strains of the bacterium. Either through mutation or gene transfer, a bacterium can, however, gain a gene that provides resistance against the antibiotic. When the antibiotic is taken again for an infection, all but the resistant bacteria die. The resistant bacteria survive and reproduce and pass their resistance gene on to offspring. This problem has been exacerbated by patients not taking a complete course of antibiotics. When they are only taken for

the first few days, the weaker bacteria are killed, leaving behind more resistant bacteria. After many generations, a new population of resistant bacteria have evolved. This is a form of natural selection. The selective pressure of an antibiotic is selecting for the antibiotic-resistant strain of bacteria to survive, reproduce and pass on the advantageous trait.

As new antibiotics have been synthesised, the environment for bacteria has changed, and the changes have killed many bacteria. The selection pressure has sped up the evolution of bacterial strains. The environment is changing quickly for many pathogens, due to climate change and use of medications. This added pressure is forcing pathogen evolution to speed up, which enables an increase in spread.

CASE STUDY

Horizontal gene transfer and natural selection

Bacteria are able to respond to selective pressures and adapt to new environments by acquiring new genetic traits as a result of mutation and horizontal gene transfer. Mutation is a modification of a gene and its function. Horizontal gene transfer is the acquisition of new genes between organisms other than from parent to offspring. Mutations usually cause small-scale changes, whereas horizontal gene transfer can cause much larger changes to a bacterium's set of traits. Some genes have transferred the code for a trait that makes the bacteria resistant to a certain antibiotic. Bacteria

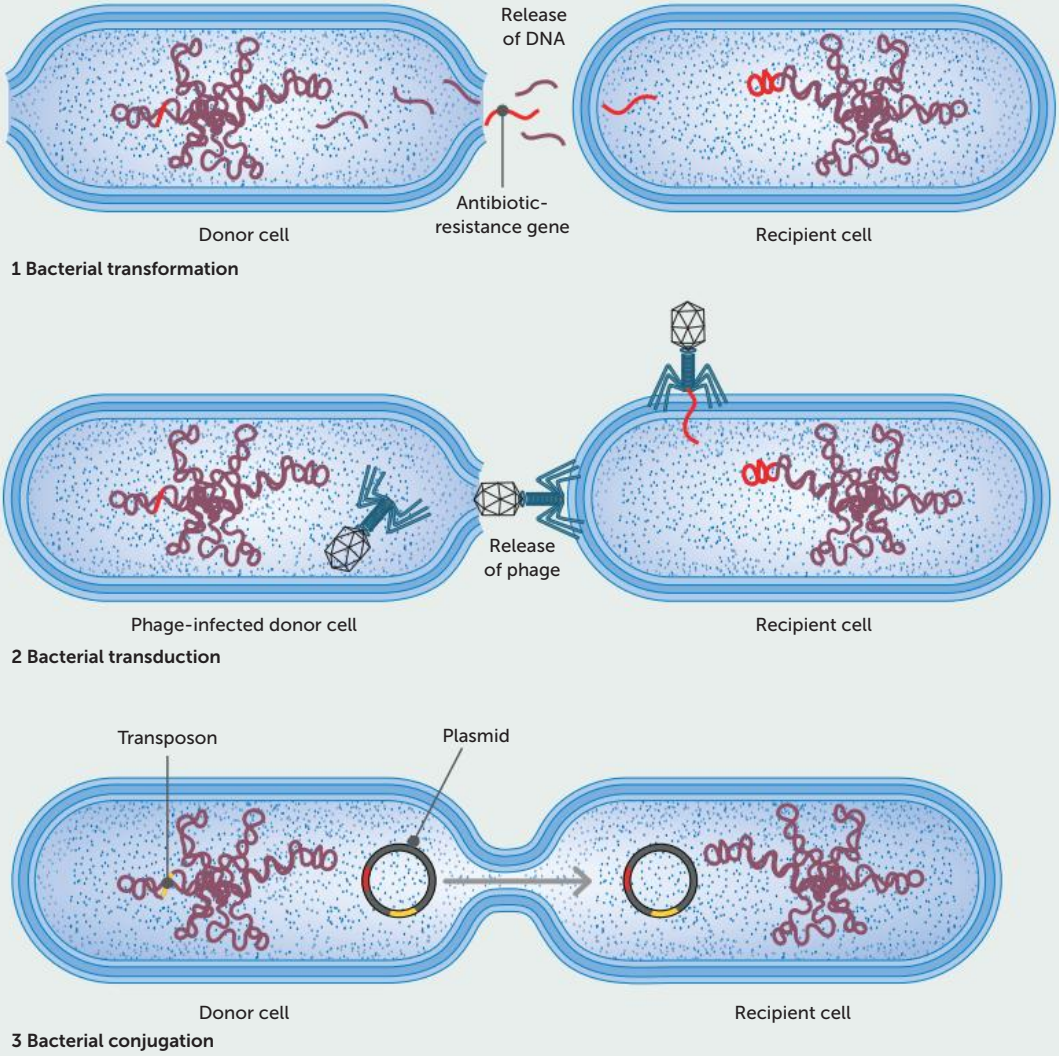

FIGURE 13.21 Three ways in which bacteria acquire DNA horizontally. In this example, the bacteria are acquiring genes that make them resistant to antibiotics.

can become resistant to an antibiotic, and over time they may acquire genes that make them resistant to multiple antibiotics, such as has occurred in the bacteria that cause TB. Resistance can build slowly through mutation over many generations, or much faster through horizontal gene transfer (see Figure 13.21).

Questions:

1. Describe three methods by which bacteria can rapidly acquire entire genes that make them resistant to antibiotics?
2. Explain how the principles of natural selection apply to the evolution of antibiotic-resistant bacteria.

Host susceptibility

Susceptibility of urban areas to epidemics and pandemics of infectious disease can be related to high population density, poor living conditions and lack of adequate **healthcare provision**. The United Nations (UN) predicts that the world's *urban* population will almost double from 3.3 billion in 2007 to 6.3 billion in 2050. More than 50% of the world's population currently live in urban areas, and this percentage keeps increasing. The reasons for migration from rural to urban areas are many, but it is common that people migrate in the hope of a better job or a better lifestyle. Most of the increase in urbanisation is occurring in developing countries, where sanitation and hygiene are relatively poor. In many cities, this has resulted in very high human densities and all of the issues associated with overcrowding. The following explore three of the main reasons why urban areas are more susceptible to infectious disease.

High population density

Urbanisation is the increase in the proportion of people living in cities. A larger number of people are living within the same limited space. With higher density living, there can be a higher contact rate between infected and uninfected individuals for any given pathogen, which means infections can spread faster. There is also an increase in interactions between animals, humans and ecosystems. An outbreak in a rural setting may be controlled quickly and easily, compared with an outbreak in an urban setting, simply because of the absence of susceptible hosts, particularly if the mode of transmission is via direct contact or close contact. Therefore, an epidemic is more likely to occur in an urban setting. An epidemic involves many individuals in a particular area being infected by an infectious disease. Rapid increases in population density can overwhelm vaccination programs, decrease herd immunity, and render a population more susceptible to outbreaks.

An example of a disease that can spread directly via close contact is influenza. Severe acute respiratory syndrome (SARS) was a major pandemic. It demonstrated the interrelatedness of urbanisation and globalisation. The first person infected with the disease lived outside a city in China. A doctor who had treated SARS patients in a hospital in that area travelled to Hong Kong and infected seven other people in a hotel there. The newly infected hosts spread the virus around the world. Therefore, the SARS pandemic started outside a city, but spread through a city, then through several other major cities, due to the ease with which a pathogen can spread through an area dense with human hosts.

Poor living conditions

Poor living conditions can lead to the spread of infectious disease, especially airborne diseases such as TB, and vector-borne diseases such as malaria. In developing countries, there is less access to clean, treated water. Sanitation facilities are inadequate. Consequently, urban slums usually have high levels of faecal matter in the water consumed by households. Waterborne diseases spread rapidly in such conditions. If electricity is affordable and reliable, then many microbes can be killed using heat treatment, but in many cities, and especially for the homeless, access to electricity cannot be relied on. Inadequate nutrition leads to weaker immune systems, increasing susceptibility to many infectious diseases. Diarrhoeal diseases are often caused by the poor sanitation associated with poverty. Though many diarrhoeal diseases are easily and cheaply treatable through oral rehydration, these diseases still claim over 1.5 million lives each year.

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Extensive animal keeping, backyard farming, and mixed production systems have also been associated with disease risks. The outbreaks of highly pathogenic avian influenza in South-East Asia have been demonstrated to be related to rice production, duck population density, and human population density. In addition to the traditional backyard poultry–keeping in urban areas, there are increasing trends of keeping small flocks of poultry in middle- and high-income urban areas in many countries. In both cases, awareness of the importance of biosecurity measures is often limited. Transmission of diseases from bird to human can occur via direct contact, as humans handle birds often when they keep them.

Opportunities for hygiene practice are limited in developing areas. With overcrowding exacerbating the situation, disease transmission can be very fast when people are not able to wash their hands, buy antimicrobial handwash or take medication to reduce symptoms that increase the chance of spread, such as a runny nose.

Lack of adequate healthcare provisions

Healthcare provisions include medicine, vaccines, bandages and other medical supplies. Healthcare services and facilities such as hospitals and health professionals are also considered to be provisions. Poor healthcare increases susceptibility to infectious disease. Poor access to medications, particularly preventative medication such as vaccination, leaves many people vulnerable to infectious diseases. Fewer individuals are able to be immunised against specific diseases. This means there is more risk of a disease spreading and a higher proportion of susceptible individuals. This is known as low herd immunity. As more people become infected and fewer people are able to access medication such as antivirals and antibiotics, little can be done to slow the spread. As more hosts become infected, pathogen numbers increase, causing populations to have a very high risk of an epidemic or pandemic.

The capacity to pay for healthcare is determined by socio-economic level. Quality healthcare is not affordable for many people living in urban settings in developing countries. Vulnerable people, such as the very young, elderly, sick or unimmunised, are particularly at risk. Diseases associated with poverty are a relatively high proportion of the diseases spreading in developing countries. Examples are malaria, which can largely be prevented with barriers and medication, and TB, which can to some extent be prevented by improving nutrition.

Key concept

Factors that affect the spread of disease include the pathogen population size, the density of the host population, the mode of transmission, globalisation, and host susceptibility. For diseases that are spread by bacteria, antibiotic resistance is also a cause for concern.

Question set 13.4

REMEMBERING

1. List the three interrelated factors that affect the spread of pathogens.
2. Describe globalisation.
3. State a definition for climate change.

UNDERSTANDING

4. ABL is fatal but has caused a low number of human deaths. Discuss the three interrelated factors that affect its spread and explain why the spread among humans has so far been low.
5. Relate globalisation to an increase in the spread of a named infectious disease.
6. Describe a series of events that climate change could trigger in a named mosquito-borne disease you have studied.
7. Explain how some bacteria have developed resistance to antibiotics.

CREATING

8. Create an infographic to illustrate how the following factors cause urban areas to be susceptible to epidemics and pandemics of infectious disease:
   - **a** high population density
   - **b** poor living conditions
   - **c** lack of adequate healthcare provisions.

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CHAPTER 13 INVESTIGATION

Developed by Southern Biological

13.1 INVESTIGATION

Infectious disease transmission: simulation of an epidemic

Background

An infectious disease is caused by a pathogen that is passed from one host to another. Spread can occur in a variety of ways, such as through direct contact with an infected individual, indirect contact via surfaces or objects touched by an infected individual, or airborne droplets that result from infected individuals sneezing, coughing or laughing. The ease of transmission of disease through airborne droplets depends on how close the infected individual and potential host are to one another, as the larger droplets disperse and settle quickly. The common cold and influenza are typically transmitted through droplets in the air.

Local health departments, WHO and Centres for Disease Control (CDCs) are responsible for monitoring infectious disease outbreaks. One of their responsibilities is to identify the source of outbreaks by tracking the routes of transmission. Over the past 100 years, these organisations have fought the spread of disease by contact tracing, sanitation improvement, vaccine development and other lines of research. Many of the infectious diseases that have historically been responsible for devastating epidemics have now been reduced or even eradicated.

Aim

To simulate a real-case scenario of infectious disease transmission and to identify patient zero

Time requirement

45 minutes

Materials

1 screw cap vial with solution (containing either 7 mL 0.001 mol L^{-1} hydrochloric acid, HCl or 7 mL 1 mol L^{-1} sodium hydroxide, NaOH)

- 15 mL phenol red indicator in a vial
- 1 plastic pipette
- Four 96-well plates (for shared use)
- 1 permanent marker
- 1 index card
- PPE: lab coats, safety glasses, disposable gloves

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Sodium hydroxide can cause severe skin burns and eye damage.	Ensure appropriate personal protective equipment is worn at all times.
Disposable gloves may pose an allergy risk.	Use a type of glove that removes allergy risk and is suitable for the chemicals being used.

Procedure

1. Collect 1 index card, 1 plastic pipette and 1 screw-cap vial containing solution. The solution in the vial represents bodily fluid. Your vial will be labelled with a number.
2. There are four 96-well plates labelled '0', '1', '2' and '3'. Locate your individual well on the class plates. This will be labelled with the number corresponding to the number on your vial.

3 Using a plastic pipette, remove some of the fluid from your vial and transfer 5 drops into your well on Well Plate 0.
4 Select a partner for your first exchange. Record their name and vial number on your index card.
5 Using your plastic pipette, transfer 5 drops from your vial to your partners' vial. Return any liquid remaining in your pipette to your vial. Replace the vial cap and mix the solution by inverting it several times.
6 Using a plastic pipette, transfer 5 drops of the fluid in your vial into your well on Well Plate 1.
7 Repeat steps 4–6 for the second and third exchanges, depositing the fluid in your vial into your wells on Plates 2 and 3, respectively. Select a different partner for each round and complete each step before proceeding to the next exchange.
8 After all exchanges have been made, your teacher will add 1 drop of phenol red; an indicator solution that will determine whether your vial has become 'infected'. Vials that turn red or pink are 'positive for the pathogen' (infected), whereas vials that turn yellow are negative; i.e. they did not become infected.

9 Report whether your vial tested positive. If so, share the names of the partners you exchanged fluids with.
10 Based on your individual results and the data from your classmates, try to identify which vial the infection spread from. Your teacher will add a drop of phenol red to each of the wells in the well plates. By observing which samples indicate a positive result in each round of transfers, you may be able to trace the spread of inspection to the original source.
11 Copy and complete Table 13.2 to identify the source of the infection. Once you have listed the positive vials and who they had exchanged fluid with, circle the numbers of the partners whose vials tested positive.

Results

TABLE 13.2 Positive vials and exchange partners

POSITIVE VIAL NUMBERS	1ST EXCHANGE PARTNER NUMBER	2ND EXCHANGE PARTNER NUMBER	3RD EXCHANGE PARTNER NUMBER

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1. Who was patient zero?
2. After the three rounds of exchanges, how many vials tested positive? Calculate what percentage of your class this represents.
3. Graph the number of students who were infected after each round.

Analysis of results

1. If the class were divided into three groups of 10 at the start of this procedure and were allowed to exchange only within their group, what would the transmission of the disease have looked like?
2. Did you know which vials were infected during the procedure?
3. Do you believe an individual who does not show any signs of a disease is capable of transmitting the disease to others?
4. What is the importance of identifying patient zero in epidemics?
5. How does this simulation differ from the spread of disease in the real world, such as the spread of COVID-19? Explain.
6. List the appropriate measures that individuals should take to limit the spread of diseases.

Taking it further

Research past infectious disease epidemics. Include information about:

- origins of the disease
- how the disease is transmitted
- typical incubation period
- signs and symptoms of the disease
- impact (i.e. death toll, cultural shifts, social effects)
- historical context
- vaccines and treatments
- preventative measures taken before, during and after.

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WS
Chapter 13 Activity sheet

CHAPTER 13 SUMMARY

- To survive, pathogens require an effective life cycle. The pathogen needs a portal of entry, to exploit nutrients, to avoid host defence mechanisms, to replicate, a portal of exit and a mode of transmission.
- Transmission can be direct or indirect.
- Direct transmission is the transfer of a pathogen from a current infected host, or other reservoir, to a susceptible future host by direct contact or droplet spread.
- Indirect transmission is the transfer of a pathogen from a reservoir to a host through non-living vectors (vehicles or inanimate objects), living vectors (living intermediaries) or suspended air particles.
- Pathogens have adaptations that facilitate their transmission by various mechanisms, including through direct contact, by contact with bodily fluids, through the air and via contaminated food, through water or by disease-specific vectors.
- The spread of a disease can be influenced by three interrelated factors: the host population density, the population of the pathogen and the mode of transmission. All three factors need to reach a certain threshold for spread to be possible.
- Globalisation is the process by which the world is becoming increasingly interconnected. Trade of goods and services, and tourism facilitate regional and global movement of pathogens. Therefore, disease is spreading globally at a much higher rate than prior to globalisation.
- Climate change is causing a rise in average air temperature and extreme weather events, including floods. Mosquitoes are more active in warmer temperatures. As previously cold areas become warmer, mosquitoes will spread further, along with the pathogens they carry. Mosquitoes exploit the extra bodies of water bodies left after floods and rains as breeding grounds, increasing the area in which they can breed and the number of offspring they can produce.
- Environmental factors contribute to the selective forces on populations, including pathogen populations. The environment is changing at a faster rate due to climate change. Factors such as increased average air temperature and habitat loss are driving forces behind rapid evolution of pathogens.
- Urbanisation is the increased trend for humans to live in cities, and it is associated with much higher density living and at times overcrowding. It can also be the cause of poorer living conditions, especially for vulnerable people living in the lower socio-economic areas of the world. These two factors, combined with limited healthcare provisions, result in higher susceptibility to epidemics and pandemics. When an outbreak occurs, it can quickly spread if people are in close contact, water is unclean and sanitation is poor, and medicine and vaccinations are inaccessible.

TABLE 13.3 Ten diseases and their life cycles

DISEASE	PATHOGEN TYPE	PATHOGEN NAME	MODE OF TRANSMISSION (most common to least common)	LIFE CYCLE SPECIFICATIONS [portal of entry, site of replication (sexual/asexual reproduction), reservoir, portal of exit]
Influenza	RNA virus	Influenza A, B or C	**Direct**, close contact via airborne droplets when infected person coughs **Indirect** via fomites	**Entry and exit** via respiratory system **Replication** inside epithelial cells in the respiratory tract; human host reservoir

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DISEASE	PATHOGEN TYPE	PATHOGEN NAME	MODE OF TRANSMISSION (most common to least common)	LIFE CYCLE SPECIFICATIONS [portal of entry, site of replication (sexual/asexual reproduction), reservoir, portal of exit]
Ross River virus disease (epidemic polyarthritis)	RNA virus	Ross River virus	**Indirect** via female mosquito vector e.g. *Aedes vigilax*	**Entry** via skin (blood feed) **Replication** in epithelial cells of mosquito vector and muscle cells of the infected human host before entering the bloodstream and then multiplying there; the reservoir is marsupials (e.g. wallabies) **Entry and exit** via two different blood feeds; exit not likely from human host; pathogen circulates through marsupials such as the western grey kangaroo
Viral diseases of honeybees, such as deformed wing virus disease	Virus	Deformed wing virus	**Indirect** via the vector varroa mite **Direct** via vertical transmission from bee to offspring	**Entry and exit** via skin (blood feeds from varroa mite vectors) **Replication** in bee
Australian bat lyssavirus	RNA virus	Australian bat lyssavirus	**Direct** contact with an infected bat's bodily fluids, through a bite or scratch	**Entry** via site of bite or other break in skin **Replication** in bat reservoir and infected human host before travelling along nerves to CNS **Exit** not likely from human host; pathogen circulates through marsupials such as the western grey kangaroo
Tuberculosis	Bacterium	*Mycobacterium tuberculosis*	**Direct**, close contact via airborne droplets inhaled into respiratory system **Indirect** if the droplets land on a dusty fomite, become disturbed and are then inhaled	**Entry** via respiratory system **Replication** in alveolar macrophages in lungs **Exit** via respiratory system through a cough or sneeze
Tetanus	Bacterium	*Clostridium tetani*	**Direct** contact between soil reservoir and deep puncture wound	**Entry** via deep wound; replication in tissue in a deep wound in the absence of oxygen; the neurotoxins travel up neurons, blocking the release of inhibitory neurotransmitters in the CNS
Crown gall	Bacterium	*Agrobacterium tumefaciens*	**Indirect** soilborne spores via a root wound, or via a vehicle such as grafting or other gardening tools **Direct** contact via infected root to wound on susceptible plant root	**Entry** via a wound on a susceptible plant root **Replication** in plant roots; bacteria transfer some plasmid DNA into the plant cell genome; the inserted DNA codes for uncontrolled plant growth; plant cells grow and divide and form galls **Exit** is via galls, through spores that drop into the soil or onto vehicles for further spread

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DISEASE	PATHOGEN TYPE	PATHOGEN NAME	MODE OF TRANSMISSION (most common to least common)	LIFE CYCLE SPECIFICATIONS [portal of entry, site of replication (sexual/asexual reproduction), reservoir, portal of exit]
Chytridiomycosis	Fungus	*Batrachochytrium dendrobatidis*	**Indirect** via soilborne/ waterborne swimming zoospores **Direct** contact via infected amphibian skin to susceptible amphibian skin	**Entry** via skin penetration (invades outer layer of skin/epithelium) **Replication** via asexual reproduction inside the thallus (zoosporangium) **Exit** is via zoospores going from the thallus in the skin of an amphibian into the water
Malaria	Protist	*Plasmodium falciparum*	**Indirect** via female *Anopheles* mosquito vector blood feeds	**Entry** via skin: blood feed by mosquito vector **Replication**: sexual reproduction between male and female gametes in the mosquito (definitive host) gut; asexual reproduction of sporozoites in liver cells; asexual reproduction of merozoites in red blood cells (the human is the intermediate host) **Exit** via skin: blood feed by mosquito vector
Phytophthora	Protist	*Phytophthora cinnamomi*	**Indirect** via swimming soilborne zoospores **Indirect** via vehicles such as car tyres and walking boots **Direct** contact between infected and susceptible roots Waterborne: zoospores can be carried by water and swim short distances to susceptible hosts	**Entry** via root tips; the mycelia threads grow on the surface and then invade the cells to gain nutrients and moisture for growth and reproduction **Replication** is on and in root cells, and is usually asexual; a few cells form a filament, filaments form mycelia threads, and a mass of threads can develop into a sporangium **Exit** from sporangia in the form of zoospores; if conditions are poor, chlamydospores are produced instead (the resilient forms of the organism); the soil is a reservoir

CHAPTER 13 GLOSSARY

Airborne droplet A tiny particle of liquid suspended in the air as part of an aerosol (solution in air) that is sneezed or coughed into air; a droplet can be suspended in an air current for a period of time before being inhaled or landing on a surface such as a table

Anaerobic bacteria Bacteria that will only germinate and multiply in hypoxic (low oxygen) conditions

Antibiotic An antimicrobial chemical that inhibits or destroys bacteria

Asymptomatic Infected but not experiencing any signs or symptoms

Blood feed The method used by female mosquitoes to ingest blood: they insert their proboscis, a tube-like mouthpiece, into the skin and blood vessel of a host to feed on

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blood; during the bite, the mosquito's saliva is transferred to the host; the saliva exiting the mosquito, or the blood being ingested by the mosquito, may potentially carry pathogens

Climate change The current climate change occurring on Earth encompasses increasing global average air and ocean temperatures, rising global sea levels, long-term sustained widespread reduction in snow and ice cover, and changes in atmospheric circulation, ocean circulation and regional weather patterns, which in turn influence seasonal rainfall conditions. The current change is thought to be mostly due to human activity, primarily the burning of fossil fuels. Already, there has been a 40% increase in heat-trapping carbon dioxide in the atmosphere

Close contact Close proximity (usually within 1.5 metres) between infected and susceptible hosts; it allows the immediate transmission of some pathogens by airborne droplets such as are produced by sneezing or coughing

Crown On a shrub or small plant, the site where the stem and roots meet; on trees, the site where a branch meets the trunk

Defence mechanism A mechanism that can prevent entry into or persistence of a pathogen within a host; it can be a physical barrier such as skin, or a non-specific cellular process such as phagocytosis

Definitive host The host in which a pathogen replicates sexually; for example, in the case of malaria, sexual reproduction of *Plasmodium* occurs in the gut of the female mosquito

Direct contact The transmitting of a pathogen through physical touch between infected host and susceptible host via skin or body fluids

Direct transmission The transfer of a pathogen from an infected host, or other reservoir, to a susceptible host by direct contact or via droplets

Distribution The location, arrangement or frequency of occurrence of an infectious disease; it describes the patterns of occurrence in geographical areas, such as 'uniform' or 'random'

Encephalitis Inflammation of the brain

Endemic A disease that is always present in a population or region

Epidemic An increase in the occurrence of a specific disease above the baseline level for a particular population; it tends to refer to larger, more serious events than an outbreak

Evolution The process of cumulative, gradual, inheritable change in a population of organisms that occurs over many generations and a relatively long time

Fever Increased body temperature

Flagellum A whip-like tail, which provides a zoospore and some other motile single cells with locomotion

Fomite A surface or non-living object carrying an infectious agent

Gall A brown, roughened lump of undifferentiated tissue on the crown of a plant (where the roots meet the stem on a small plant, or where a branch meets the trunk of a tree); it looks tumour-like

Gametocyte An underdeveloped male or female sex cell; the form of *Plasmodium* that is ingested by mosquito vectors during a blood feed on an infected human

Globalisation The process by which the world is becoming increasingly interconnected as a result of massively increased trade, economic, travel and cultural exchange

Healthcare provision The provision of medicine, vaccines, bandages and other medical supplies, healthcare services and facilities such as hospitals and healthcare professionals

Impact on the host The signs and symptoms of an infectious disease, and its effect on tissue structure and function; tissue damage or abnormal function may be due to the replication of the pathogen or to toxins produced by the pathogen; death is a possible impact in some cases

Indirect transmission The transfer of a pathogen from a reservoir to a host through vehicles (inanimate objects), living vectors (living intermediaries) or suspended air particles; indirect transmission may require one or more steps

Intermediate host The host in which a pathogen replicates asexually; in malaria, this occurs in the liver and red blood cells of a human

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Interrelated factors Factors that affect or are affected by one another

Keratin A strong, stable structural protein found in skin, hair, horn and nails

Life cycle The cycle a pathogen goes through to survive and reproduce; it includes invasion of the host, exploiting a nutrient-rich area of the host, avoiding host defence mechanisms, replicating, exiting, and transmitting to new hosts

Lysis The process of a cell bursting (verb: to lyse)

Macrophage A white blood cell that can perform phagocytosis on microbes such as pathogens, by engulfing them (see **endocytosis** in Chapter 12) and destroying them with the use of enzymes

Merozoite A relatively mature form of the *Plasmodium* pathogen; it is the form that develops in the liver of a human host, exits the liver, and invades red blood cells, spreading among them until some develop into gametocytes

Mucous membrane A mucus-secreting membrane that lines the respiratory, digestive, excretory and reproductive tracts (note the different spelling of the mucous membrane and the mucus it secretes)

Mycelial thread Also known as a hypha, a mycelial thread grows on the surface of infected roots, absorbing nutrients; it is highly branched and can be observed by the naked eye, growing on rotten plant roots

Outbreak A sudden, unexpected increase in the prevalence of a particular disease above the baseline level for that population; it could be a single case of a contagious disease in a small community

Pandemic A disease that has spread rapidly throughout the world; an epidemic that has crossed international borders

Phagocyte A cell that is capable of phagocytosis; it can be a macrophage or a neutrophil

Phagocytosis The process of engulfing and destroying a microbe

Population density The number of organisms of the same species living in a particular area at a specified time

Portal of entry The site where a pathogen can enter a susceptible host; includes mucous membranes lining the respiratory tract or the gastrointestinal tract, or a break in the skin of an animal or the bark of a plant

Portal of exit Many pathogens exit the host in the same way that they enter; other portals of exit include excretion from the digestive system or via the reproductive system

Reservoir An organism (such as a wallaby) or habitat (such as soil) in which a pathogen can reside, and sometimes replicate, prior to entering a susceptible host; a reservoir is somewhere in which the pathogen does not go extinct

Sign An objective and measurable experience of a pathogen host that is directly observable: elevated body temperature, breathing rate, pulse rate and/or blood pressure are important 'vital' signs of disease

Sporozoite The tiny infectious cell form of a parasite (such as *Plasmodium*); it is often the infective agent that enters the host; it is a relatively immature form of a pathogen

Spread To transmit a pathogen to susceptible hosts over a wider area and into new populations

Susceptible host An organism that is vulnerable to developing infection when invaded by germs; very young children, older people, people who are ill or who are receiving particular medicines that reduce their immunity, people with long-term health conditions like diabetes, and those who are physically weak due to, for instance, malnutrition or dehydration can be particularly susceptible

Symptom A subjective experience felt by a patient, such as nausea or pain

Threshold A certain level that must be exceeded for a result to be produced (the spread of a pathogen is reduced unless the host population reaches the threshold value)

Transmission Transport of a pathogen from an infected host or a reservoir to a susceptible host

Tubercle A small, round structure made of cells that is produced as a result of an infection

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Urbanisation The increase in the proportion of people moving from rural areas to live in towns and cities

Vector In reference to diseases, an agent that transmits pathogens from one host to another; in genetics, a vehicle used to transfer DNA sequences from one organism to another

Zoonotic disease A disease that animals pass to humans; an infection that is naturally transmitted between vertebrate animals and humans

Zoosporangium (thallus) A roughly spherical, smooth-walled growth; inside the thallus, asexual reproduction occurs, producing new zoospores; the thallus contains a plug that is removed once the thallus matures, releasing the zoospores into water

Zoospore A spore with a flagellum; one of several forms of a fungal or protistan organism

CHAPTER 13 REVIEW QUESTIONS

Remembering

1 For a pathogen, state the purpose of a host.
2 Describe the generic life cycle of a pathogen.
3 List three interrelated factors that can affect the rate of spread of a pathogen.

Understanding

4 Summarise the life cycle of the pathogen that causes chytridiomycosis.
5 Describe the site/s and form/s of the parasite *Plasmodium* during;
   a sexual reproduction
   b asexual reproduction.
6 Name two diseases with more than one mode of transmission and describe the modes.

Applying

7 Discuss the possible impacts of global climate change on the distribution of mosquito-borne diseases.
8 Describe two major differences between the pathogen that causes malaria and the pathogen that causes Ross River virus disease.
9 Provide a rationale for spending money on biosecurity to prevent the varroa mite from entering Australia.
10 Differentiate between an epidemic and a pandemic.
11 Explain why tuberculosis spreads easily in urban areas of poor countries.

Analysing

12 Compare crown gall and tetanus, using the following terms:
   a pathogen type
   b pathogen forms
   c aerobic
   d spores.

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Evaluating

13 Given the increase in antibiotic resistance in recent years, discuss whether we should restrict the use of antibiotics to only those people with a life-threatening illness.

14 Consider your knowledge of infectious disease and evaluate urbanisation, listing two advantages (pros) and two disadvantages (cons) for humans.

Creating

15 Design an experiment to test the effectiveness of a new antibiotic on a specific bacterium.

PRACTICE EXAM QUESTIONS

1 A pandemic is most likely to arise from a new influenza virus strain that:
- **A** spreads easily among humans
- **B** causes a high mortality rate in humans
- **C** cannot replicate in humans
- **D** has the same protein coat as an existing strain.

[Q16 2018 SCSA]

2 A pathogen that infected plants has cells with a true nucleus, mitochondria and a cell wall made of chitin and is therefore a:
- **A** bacterium
- **B** fungus
- **C** protist
- **D** virus.

[Q17 2017 SCSA]

3 In the course of an influenza epidemic, the number of susceptible hosts will:
- **A** stay the same
- **B** increase
- **C** decrease
- **D** fluctuate.

[Q29 2017 SCSA]

4 An outbreak of a serious new strain of influenza occurs on a cruise ship at sea. The best method of preventing the influenza from spreading to populations on land is to:
- **A** keep all people on the ship until everyone has recovered
- **B** send crew members ashore to obtain antiviral medication
- **C** disinfect all eating and recreational areas on the ship
- **D** bring in medical personal to vaccinate people on the ship.

[Q8 2016 SCSA]

5 Many strains of bacteria that cause diseases in humans are evolving resistance to antibiotics. Explain how a disease-causing strain of bacterium can evolve resistance to an antibiotic used to treat the associated disease. (4 marks)

[Q34e 2016 SCSA]

6 Describe how malaria is transmitted from an infected person to an uninfected person. (4 marks)

[Q35b 2018 SCSA]

7 Malaria is distributed mainly near the equator, where it is warm and has relatively high rainfall. Unlike malaria, tuberculosis occurs throughout the world. Explain why tuberculosis is much more widely distributed than malaria. (5 marks)

[Q35e 2018 SCSA]

8 Discuss how population density can influence the susceptibility of an urban area to an influenza epidemic. (5 marks)

[Q38 2018 SCSA]

9 Discuss how the provision of healthcare can influence the susceptibility of an urban area to an influenza epidemic. (5 marks)

[Q38 2018 SCSA]

10 Discuss how phytophthora dieback disease spreads and the management strategies that can be used to control the spread of this disease. (10 marks)

[Q39 2018 SCSA]

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14 PATHOGEN MANAGEMENT STRATEGIES

CHAPTER 14 CONTENT

By the end of this chapter, you will have covered the following material.

STARTER QUESTIONS

1 Recall one or two diseases you have studied in Chapters 12 and 13. Can you give examples of how Western Australia attempts to control the spread of those infectious diseases locally and across the state?
2 Can you describe how Australian authorities have kept many infectious diseases outside our national borders?
3 Of the pathogens that you have studied so far, which is the most difficult to manage in terms of controlling spread, and can you explain why?

SCIENCE UNDERSTANDING

» management strategies are used to control the spread of infectious diseases, including
  – quarantine
  – immunisation – herd immunity
  – disruption of pathogen life cycle
  – medications – antibiotics and antivirals
  – physical preventative measures

SCIENCE AS A HUMAN ENDEAVOUR

» contemporary models can project the spread of disease and simulate the effects of possible interventions. Supercomputing has enabled models to predict the relationships between epidemic frequency and location, and factors such as population size, environmental change, persistence and antibiotic resistance
» international cooperation and communication are needed to evaluate the risk of the spread of disease, including the emergence of new viral diseases
» quarantine measures protect Australia's agriculture industry and environment against the influx of disease-carrying materials and organisms in the face of increasing global trade and travel

ATAR Biology Syllabus, Government of Western Australia, School Curriculum and Standards Authority

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14.1 WHY DO WE NEED PATHOGEN MANAGEMENT STRATEGIES?

Growth in the number of **emerging diseases**, speed of transmission, and distribution of familiar diseases has increased rapidly in the last few decades. Due to a boom in low-cost international flights, **outbreaks** of disease that could previously have been contained within small areas or communities can now spread quickly and become global incidents. Throughout history, some diseases have not been eradicated, but have remained resistant to medical **treatment**. Some have persisted after hundreds of years of infecting our societies.

Emerging diseases fall into one of the following three categories:

- diseases that have recently appeared in a population
- diseases that have occurred previously but until recently have affected only small numbers in isolated places
- diseases that have occurred previously but only recently have been associated with a newly identified pathogen.

Emerging infectious diseases
Investigate several emerging infectious diseases at this website.

Examples of emerging diseases are COVID-19, ebola, AIDS, and the re-emerging diseases malaria and tuberculosis (TB). Understanding the factors that contribute to the spread of disease within a population, and globally, is important in managing disease outbreaks. Management of spread relies on communication and collaboration between countries, organisations and communities. Interventions can then be designed to target factors that prevent further spread of disease. Understanding the life cycle of a pathogen can help scientists work out how to prevent and control the spread of the disease it causes. Factors that can be targeted are:

- the mechanism of transmission
- environmental factors (such as climate)
- characteristics of the infected population (such as levels of **immunisation**).

Disease management is a coordinated response involving **prevention**, control and treatment, and is a response that is specific for each infectious disease.

Prevention and early detection are by far the most effective strategies, because most emerging diseases are caused by viruses for which we currently have no **vaccines**. Prevention includes the prevention of the transmission of a disease, of the onset of disease **signs** and **symptoms**, and of the impact of the disease on the environment or society. It involves a range of simple to complex measures, such as washing hands, making health services accessible, and utilising predictive computer modelling. Practices that prevent the spread of disease include hygiene practices, **quarantine**, **vaccination**, public health campaigns, use of pesticides, and genetic engineering.

Control measures are a set of strategies that reduce the incidence and duration of a disease. **Management strategies** may help control the spread of a disease, which may result in localised elimination of that disease, and may ultimately lead to its global and permanent eradication. They involve meticulous preparation for and rapid responses to outbreaks at community, state, national and global levels. Australia has strict border control and quarantine strategies for the purpose of limiting an outbreak. When an outbreak occurs, response teams plan and carry out strategies that aim to minimise spread of a disease. The World Health Organization (WHO) has response teams with strategies aimed at:

- prevention (once the outbreak has happened)*
- anticipation
- early detection
- containment
- control
- eradication.

*Control of a disease also involves preventative measures, but these strategies are being applied after an outbreak.

Treatment involves providing healthcare, such as medication and vaccination, at the right time and cost. Antimicrobial medications are crucial for managing certain diseases, but many medicines get distributed according to a community's ability to afford them. Following treatment, if the **host** no longer has the pathogen present, or signs or symptoms of disease, then they are said to be cured.

Some **epidemics** have well-established control measures, yet they remain a threat for many individuals for a variety of reasons. Influenza remains a threat because the pathogen rapidly changes strain. TB bacteria have evolved to become resistant to **antibiotics.** Many people can't afford the testing needed to diagnose a disease, or its treatment. For many diseases, eradication awaits financial help, especially to increase health provisions.

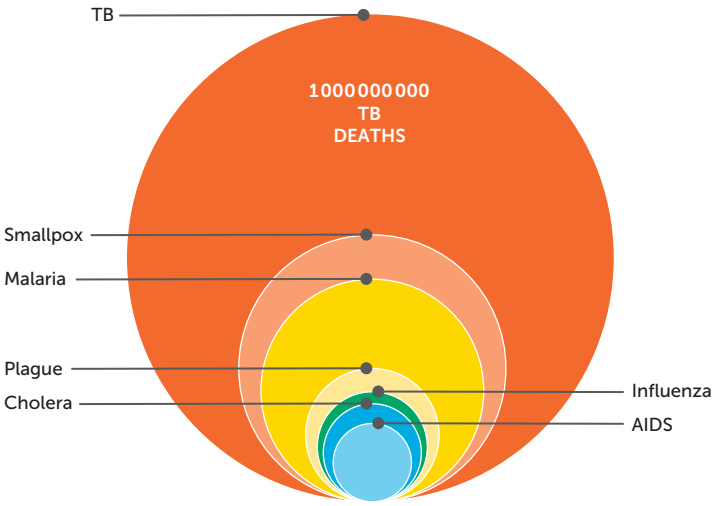
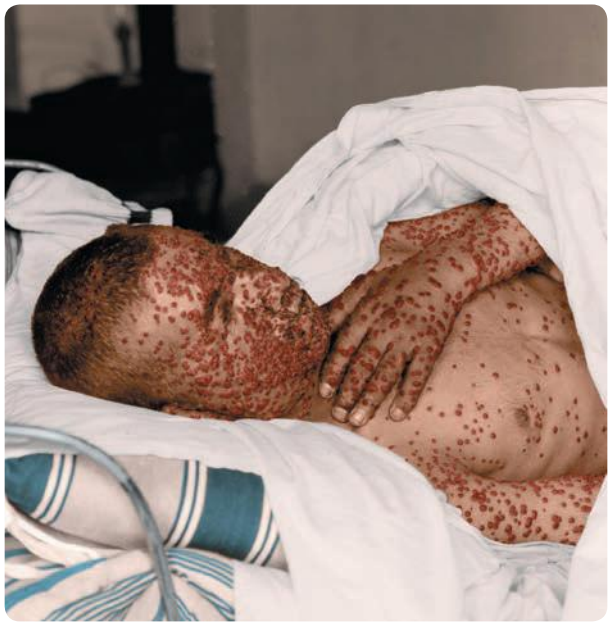


FIGURE 14.1 Some infectious diseases have not been eradicated, even after hundreds of years of infection.



Alamy Stock Photo/Science History Images

FIGURE 14.2 A person infected with smallpox. Vaccination efforts meant that the disease was declared to have been eliminated in 1980.

Epidemiology is the study of the occurrence of disease in populations. **Epidemiologists** are professionals who work to prevent or minimise the impact of diseases in the population. Their work may include such activities as identifying outbreaks, determining the effectiveness of a vaccine, and

calculating the cost effectiveness of various means of controlling disease transmission. Occasionally, epidemiologists act as detectives, tracking down the cause of an emerging disease, determining its reservoir and mode of transmission, and helping organise healthcare workers to bring the disease under control. Epidemiologists aim to describe patterns of disease, identify causes of disease, and provide data for management of a disease. They analyse data gathered about **notifiable diseases**. Notifiable diseases are communicable diseases that, if diagnosed, are required to be reported to a state government healthcare working group. In Australia, the Communicable Diseases Network Australia team have agreed on a list of communicable diseases that require reporting by the public. Data on notifiable diseases are analysed under the Commonwealth's National Notifiable Diseases Surveillance System. If outbreaks occur that put communities at risk, control measures will be executed. Information can then be sent to international bodies such as WHO for global surveillance and management. International cooperation and communication are needed to evaluate the risk of the spread of an infectious disease, including emerging diseases.

In 2018, 27 980 notifiable infectious diseases were reported in Perth. Diseases of concern that are on the rise include sexually transmitted infections, measles, influenza and varicella. In addition, although the benchmark for fully immunised 1-year-olds is 95 per cent for achieving community protection, fewer than this number were immunised that year (93.7%). This is a cause of concern for epidemiologists, health professionals and government officials and the wider community who wish to minimise outbreaks of infectious diseases.

Key concept

Epidemiologists develop management strategies which focus on the prevention, control and treatment of disease, in order to minimise the spread of outbreaks.

Question set 14.1

REMEMBERING

1. What are the three different categories of emerging diseases?
2. Recall the terms that best describe the following:
   - a. the study of the occurrence of a disease in populations
   - b. a coordinated response involving prevention, control and treatment
   - c. a disease, usually viral, that has recently appeared in a population.
3. List the criteria for someone to be identified as 'cured' of an infectious disease.
4. How does understanding the life cycle of a pathogen help to prevent and control disease?

UNDERSTANDING

5. Explain why an international team is required for managing infectious diseases. Why can't Australia manage diseases effectively without international communication and cooperation?

14.2 STRATEGIES THAT CONTROL THE SPREAD OF PATHOGENS

Management strategies should be utilised before and during an outbreak, depending on the pathogen: quarantine, immunisation, disruption of pathogen life cycles, medications (antibiotics and **antivirals**) and physical preventative measures. There is not just one method for disease management. We will explore generic management strategies, and also specific advice for specific diseases.

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Quarantine

Quarantine is a period of isolation undertaken by potentially infected individuals to prevent the spread of a contagious disease. Organisms suspected of carrying a disease are isolated from local, susceptible populations until at least the incubation period is finished, and clinical signs and symptoms have passed and/or test results are negative. Australia has strict quarantine laws that prohibit the entry of items that may carry an infectious animal or plant disease. When products are potential **carriers** of pathogens, **biosecurity** officers enforce a compulsory period of isolation of the products. Biosecurity is a set of strategies that support the prevention of, response to and recovery from diseases that affect our economy, environment and health. Biosecurity in Australia has maintained a disease-free status for many of the world's diseases. Our geographic isolation plays a major role in maintaining this status, but globalisation has diminished the geographical advantage. With nearly 60 000 kilometres of coastline offering a variety of pathways for exotic pests and diseases, the Department of Agriculture screens, inspects and clears the millions of people, mail parcels, baggage, ships, animals, plants and cargo containers entering Australia every year using X-ray machines, surveillance and detector dogs. Quarantine is a practice used by our biosecurity officers to stop goods and individuals who have been exposed to infectious diseases from carrying that disease into healthy, susceptible populations. It is used to counter the threat of spreading disease via the movement of infected individuals.

If harmful diseases enter Australia, they can cause huge financial losses for many industries, especially agriculture. It is also costly to attempt to bring a disease under control once it has entered. The cost of quarantine is much less than the cost to industry that accompanies an outbreak. Northern Australia has implemented extreme quarantine rules because of its proximity to the South-East Asia and Pacific region. The Northern Australia Quarantine Strategy is implemented by the Australian Quarantine and Inspection Service (AQIS) to monitor the comprehensive quarantine regulations for the coastline from Cairns to Broome, including the Torres Strait.

Quarantine was first used during the 14th century to stop the spread of the bubonic plague. Ships arriving into Venetian ports had to anchor just outside the port for 40 days before passengers could disembark. The modern term 'quarantine' derives from the Italian word *quaranta* meaning 'forty'.

Captains of planes and ships carrying passengers collaborate with biosecurity officers because they are required to report passengers displaying symptoms of certain infections. In exceptional circumstances, intensified quarantine measures may be implemented at airports to try to prevent the spread of disease by air travel. For example, in 2009 during the H1N1 influenza (swine flu) **pandemic**, thermal imaging cameras were used at airports to try to detect people with a fever who might have the disease.

On 12 March 2020, COVID-19 was declared a pandemic by WHO. From 28 March 2020, passengers arriving in Australia from overseas were subject to the Australian Government's mandatory quarantine period of 14 days. Passengers were provided with suitable accommodation, usually a hotel, to stay in during this period. Those in quarantine had to remain in the allocated accommodation until they were medically cleared to enter the Australian community.

Goods brought into Australia on passenger planes and commercial shipments are also inspected for high-risk items, such as meat or plant products. These products can then be stopped from entering the country. Australia has particularly strict quarantine laws because of the potentially devastating impact of imported pathogens on our unique flora and fauna. As an island, protecting our borders from imported pathogens and pests is easier than in many other parts of the world.

To protect Australia's agriculture industry and environment against the influx of disease-carrying materials and organisms, the following measures are undertaken:

1. inspection of all material brought into Australia; confiscation of suspect items
2. materials and organisms, particularly plants, that display the impact of an infectious disease are destroyed or kept in quarantine stations
3. monitoring for **vectors** entering Australia, such as the varroa mite; use of biotechnology to reliably identify species.
4. Northern Australia on high alert for infectious disease, due to its close proximity to South-East Asia.
5. Any items such as tools are treated before movement between regions.

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Queensland fruit fly
For updates on the Queensland fruit fly epidemic, refer to this website.

WA has remained relatively free of pests and diseases that would adversely affect our agricultural industries and environment. In March 2020, Queensland fruit fly, a serious pest of many fruits was detected in Perth. Larvae develop within the fruit, feeding from it and ruining the harvest. When the adults emerge, females penetrate fruit to lay their eggs. The wound this makes allows fruit-rotting bacteria to enter. A quarantine area has been set up, along with a baiting and trapping program, to manage the outbreak. WA farmers rely on quarantine to keep their crops disease-free, enabling them to export their crops to worldwide markets. The Department of Agriculture and Food, through Quarantine WA, enforces strict biosecurity legislation on imports. They inspect the following potential carriers:

- new or used machinery that is used in association with agriculture, animals, mining or earth moving
- animals or animal products
- bees, honey and other hive products
- animal feed of plant origin other than processed or manufactured feed for dogs, cats and fish (including hay, straw, fodder, seed and other grain used for animal feed)
- plants or plant products, including flowers, cuttings, bulbs, fruit and vegetables (excluding canned or cooked plant products)
- seeds
- absorbent pet litter derived from plant material
- soil
- plant-growing media and landscaping material such as potting mix, wood-chips and mulch
- cargo containers
- containers used for, or in connection with, agricultural products
- containers used to transport animals (other than dogs and cats)
- containers of live fish, including all contents and the fish
- vessels and vehicles.

Myrtle rust: dodging the bullet?
Read about the disease in this article.

SCIENTIFIC LITERACY

Quarantine in action: Department of Biodiversity, Conservation and Attractions

WA and South Australia have so far successfully prevented the entry and spread of a plant fungus disease – myrtle rust – even though the disease has spread through all other states and territories. Learn about it through the weblink.

1 Explain the potential impact on our environment if it did spread to WA.
2 What role does quarantine play in preventing the disease from entering our state?

Question set 14.2a

REMEMBERING

1 Recall the term for the following:
   a a set of strategies that support the prevention of, response to and recovery from diseases that affect our economy, environment and health
   b a period of isolation to prevent the spread of a contagious disease.
2 List five potential carriers of disease inspected by biosecurity officers in Australia.

UNDERSTANDING

3 Explain why the northern half of Australia conducts more biosecurity surveillance and quarantine than the southern half?

Immunisation and herd immunity

Immunisation is the act of protecting someone from disease by the use of a vaccine, and also describes the process of developing resistance to a specific disease. A vaccine stimulates active immunity, which is the production of specific **antibodies** in a susceptible host during its response to a specific pathogen, and it promotes the formation of memory cells. A serum-type vaccine can be used, but this leads to passive immunity and no formation of antibodies or memory cells. Antibodies are special proteins produced by white blood cells. They react with and help make pathogens harmless. Antibodies are also known as immunoglobulins, and they are produced by specialist white blood cells called B cells. Vaccination is the administration of a vaccine into the bloodstream to cause immunity, usually by injection. Immunisation is what happens in your body after you have a vaccination. The vaccine stimulates your immune system so that it can recognise the disease and protect you from future infection (i.e. you become immune to the infection).

Vaccines help develop immunity by inducing a response to the part of a pathogen called an antigen. During infection, antigens, which exist on the surface of pathogen, trigger a response. A vaccine also triggers the response (production of antibodies), without the presence of the pathogen in its typical form. The pathogen may be dead or attenuated; therefore, the immune response is initiated without developing the disease. Minor symptoms may be experienced instead of the full effects of a disease, but most importantly, the host develops a supply of specialist 'memory' cells that the body can use as a defence if infected with the same pathogen.

Vaccines consist of a dead, weakened or inactive form of a pathogen. For example, the vaccine may be a pathogen that has had DNA or RNA removed, preventing the **replication** of the pathogen but still supplying the antigen required for an immune response by the recipient. Vaccines contain antigen components from an infected organism. If the antigen components stimulate an immune response (but not disease), they can protect against subsequent infection by that organism.

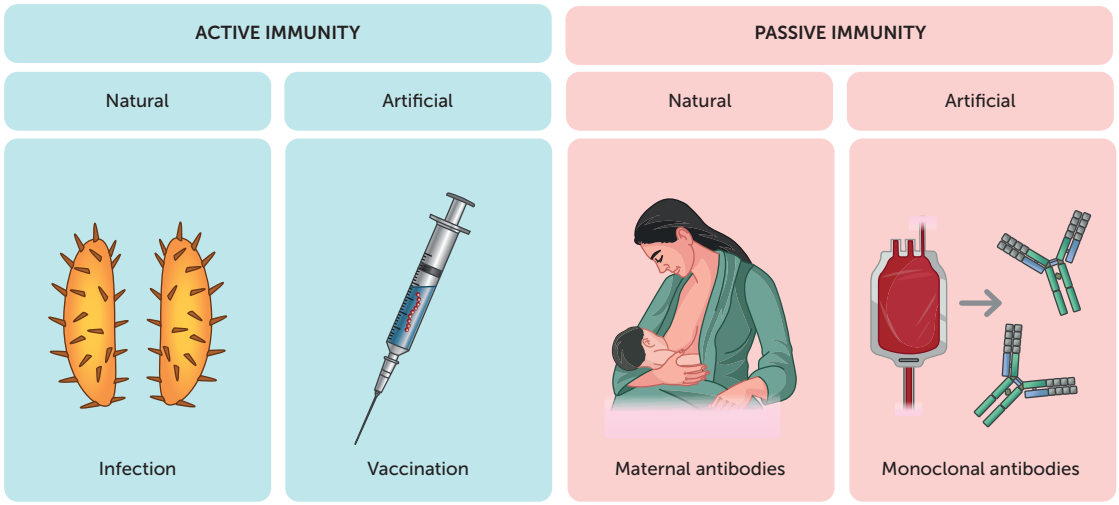

FIGURE 14.3 Examples of active and passive immunity

What is herd immunity?

When a high enough proportion of a population, the threshold proportion, is immune to an infectious disease, the fraction who are not immune are to a large degree protected from transmission. This is known as **herd immunity**. Herd immunity can stop a disease from spreading, and it prevents outbreaks. When a high threshold percentage of the population is vaccinated, it is difficult for infectious diseases that are contagious to spread, because there are not many people who can be infected. For example, if someone with measles is surrounded by people who are vaccinated against measles, the disease

cannot easily be passed on to anyone, and it will quickly disappear again. This is because there will too few susceptible hosts for the pathogen to be transmitted to. The lack of susceptible hosts provides protection to the vulnerable people in a community, such as people with a compromised immune system, new-born babies and the elderly. If a certain threshold of people have been immunised, then the few who have not have a very low chance of becoming infected. This is an effective management strategy for preventing epidemics.

Herd immunity does not protect against all vaccine-preventable diseases. The best example of this is tetanus, which is infectious but not contagious. The bacteria are transmitted from the environment (soil), not from other people who have the disease. No matter how many people around you are vaccinated against tetanus, it will not protect you from tetanus.

Principles of herd immunity

1. A critical (high enough) proportion of the host population becomes immune to a specific disease.
2. Immunity is usually by an artificial vaccine (or can be gained naturally by recovery from disease) causing the formation of specific antibodies and memory cells against a specific pathogen.
3. This limits the spread of the disease, because there are too few susceptible people to sustain the spread. The pathogen cannot reproduce at a high enough rate to sustain its population.
4. Infected hosts carrying the pathogen are more likely to come into contact with immune individuals, reducing the possibility of transmission and reducing the risk for susceptible people.
5. The higher the proportion of immune individuals, the greater the protection.
6. It protects people who cannot be vaccinated, such as people with an anaphylactic reaction to a vaccine ingredient (which is rare), and pregnant women or those undergoing a treatment that suppresses their immune system (in the case of vaccines containing live viruses).
7. The proportion of the population who need to be immune to create herd immunity depends on the **virulence** of the pathogen.

Figure 14.4 demonstrates why herd immunity occurs when the proportion of a population who are immune to a disease reaches a high enough level. Infected individuals (orange) are only able to spread the disease when they come into contact with susceptible individuals (black). When there are enough immune individuals (blue), the chance of an infected individual coming into contact with a susceptible individual is so low that the disease cannot spread. For herd immunity to prevent the spread of disease, a high proportion of the population needs to be immune. The exact proportion depends on the virulence and **infectivity** of a particular disease. Some individuals have health conditions that mean they cannot be immunised, and they have to rely on herd immunity for protection from infection.

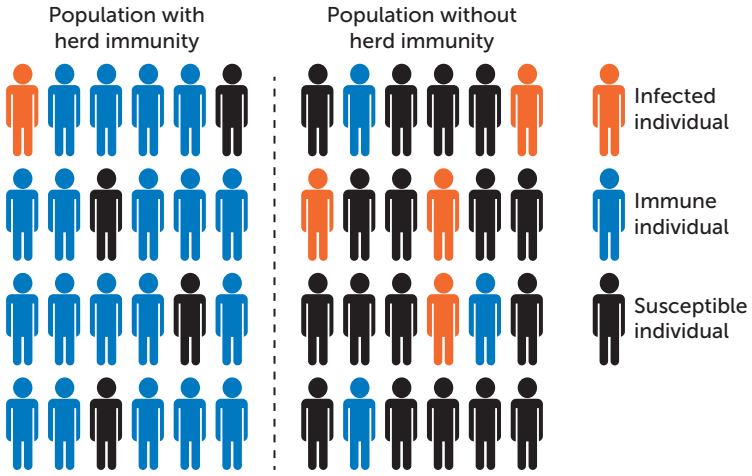

FIGURE 14.4 Principles of herd immunity

There are concerns about vaccines. Side effects can be experienced by the recipient. However, significant harmful side effects are rare.

In Australia, children are routinely vaccinated against a large number of infectious diseases, including hepatitis B, pertussis, measles, tetanus and poliomyelitis. Groups that are at high risk of infection, such as people with complex health issues or chronic illnesses, may need additional vaccinations. As new vaccines are developed, there are immunisation programs against an increasing number of diseases, with evident benefits to health. Figure 14.5 shows the rates of infection of *Haemophilus influenzae* and meningococcal C after the introduction of vaccines against these pathogens.

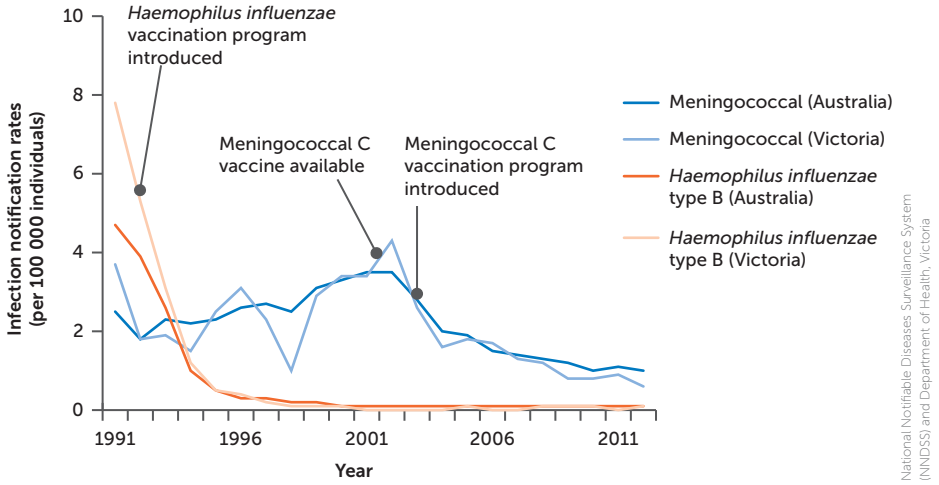

FIGURE 14.5 Rates of *Haemophilus influenzae* type B and meningococcal infection since the introduction of vaccines against these diseases

Herd immunity

14.1 APPLICATION

Read the information and watch the video in the weblink to find out more about herd immunity.

1. How does vaccination help you?
2. How does vaccination help others?
3. Describe the principles of herd immunity.
4. Why does vaccination not help with tetanus?

Herd immunity
Explore the principles of herd immunity and watch the video.

Key concept

Quarantine and immunisation strategies aim to reduce the spread of infectious disease by isolating potential carriers away from the population (quarantine) and reducing the number of infections of a population (immunisation and herd immunity).

Question set 14.2b

REMEMBERING

1. Define:
   - a immunisation
   - b vaccination
   - c antibodies.
2. Outline the principles of herd immunity.

UNDERSTANDING

3. Apply the principles of herd immunity to the control of influenza in Australia.
4. Copy and complete the flow diagram in Figure 14.6 (page 478) to describe, in detail, the sequence of events a community experiences in order to gain herd immunity. One of the steps has been completed for you as an example.

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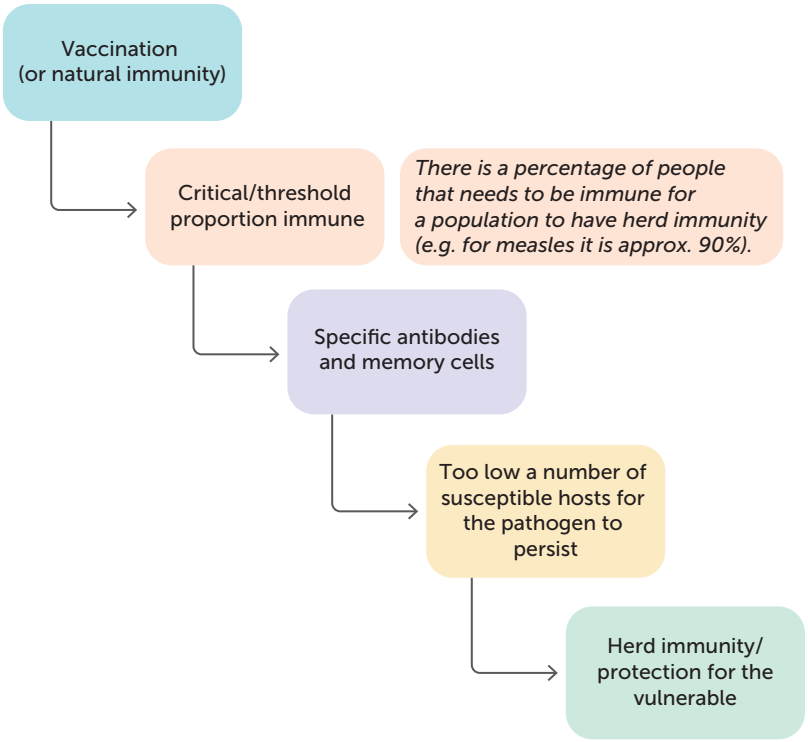

FIGURE 14.6 The sequence of events a community experiences in order to gain herd immunity

Disruption of a pathogen life cycle

Understanding the life cycle of a pathogen can help scientists work out how to prevent and control the spread of the disease it causes. The life cycle of each pathogen is unique, but it generally involves a reservoir, a portal of exit from an infected host, a mode of transmission, a portal of entry, replication and a susceptible host. Many of these factors can be targeted in order to break the cycle and reduce the survival of the pathogen. Some factors that are targeted include the mechanism of transmission, mechanism for entry, replication stages, **persistence** in a reservoir, portal of exit and immunity in host.

Replication is the process of producing new pathogens from old pathogens. Viruses replicate by taking control of host cell replication enzymes. Bacteria replicate by binary fission, a form of asexual reproduction. Fungi and protists may reproduce via sexual or asexual reproduction.

Persistence refers either to the ability of a pathogen to survive for long periods in reservoirs, or how long the pathogen remains viable outside the host.

If pathogen populations stop transmitting, infecting, replicating, persisting, or gaining nutrients, or can't survive against the immune system of hosts, then the disease may stop spreading. When one of three interrelated factors influencing the spread of disease is missing, spread stops. This can lead to elimination or even eradication of a disease. The three factors needed for disease spread are:

1. a susceptible host in sufficient density
2. growth of a virulent pathogen population
3. transmission.

Epidemiologists have been studying the life cycle of *Plasmodium* for decades with the intention of targeting a stage in the life cycle as a measure of control. Complex analysis of the biology of the organism's life cycle is crucial to meeting the aim of stopping its spread.

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Plasmodium causes the disease malaria. This protistan pathogen requires two types of hosts in its life cycle; one of them, the female *Anopheles* mosquito, also transmits the pathogen from infected to uninfected hosts. The mosquito gut is where *Plasmodium* reproduces sexually. Thus, there are two essential hosts involved in the life cycle: the **intermediate host** (the human), and the mosquito vector that also plays the role of **definitive host** (the host within which the adult form of the parasite produces gametes).

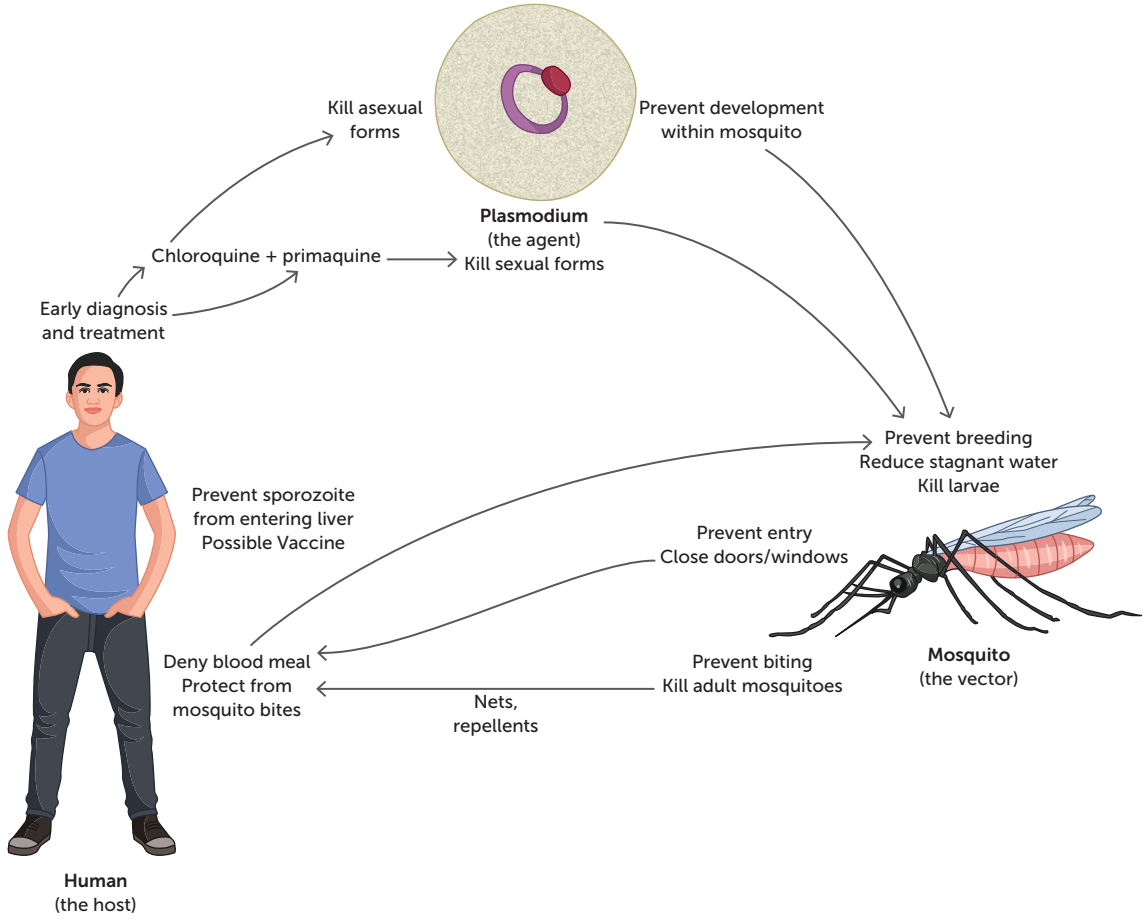

FIGURE 14.7 Control strategies at the various stages of the life cycle of *Plasmodium*

Mosquitoes are a vector for the transmission of *Plasmodium*. They have been targeted as a control measure. However, adult mosquitoes are highly active and have shown resistance to insecticides, making population control difficult. It is therefore important to target their larvae. The pathogen has also shown resistance to the anti-malarial drugs. Each control target is promising, but it also has issues. Targeting the human host through early diagnosis and the use of medication has potential, but poverty, low access to health provisions, and poor education have prevented this approach from breaking the cycle. Killing both the asexual and sexual forms of the parasite has been made possible, but drug resistance has evolved. Killing the mosquito vector with insecticide has been effective in large areas of the world, but insecticide resistance has evolved. Lastly, prevention of transmission via blood feeds through using barriers to infection (such as bed nets, clothing, closed windows and insect repellent) has reduced the rate, but not all people are diligent or educated about the effectiveness of these simple measures.

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If all humans could somehow be protected from infections for a few months at a time (e.g. through vaccination), the malaria parasite would eventually die out, because all malaria-carrying mosquitoes naturally have a short life span. However, this vector-transmitted disease is not currently eradicable because so far we have no consistently effective eradication measures.

Travellers to areas where malaria is **endemic**, and vulnerable individuals living within those areas (e.g. pregnant women, patients with complex health issues, patients with organ failure), should be started on chemoprophylaxis (preventative treatment with drugs) against malaria, before travel in the case of travellers. The chemoprophylaxis involves taking antimalarial drugs every day (or weekly in the case of some antimalarial drugs), so as to suppress malaria.

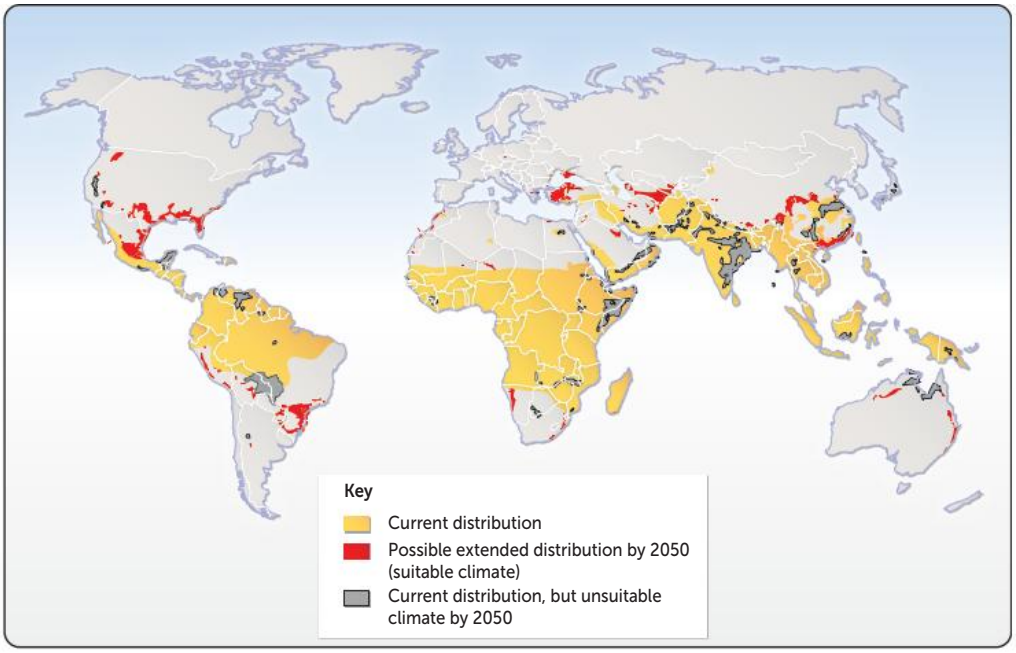

FIGURE 14.8 Map showing the predicted changes in distribution by 2050 of *Plasmodium falciparum* malaria, based on modelling data

Stopping mosquito-borne disease
This interactive investigates the life cycle of mosquito vectors with the aim of stopping the spread of a disease.

In 2002, scientists succeeded in sequencing the *P. falciparum* genome, which has allowed researchers to better understand ways to target it. For around three decades, scientists have been making progress with a number of vaccines. The vaccine that was trialled in Malawi, Ghana and Kenya is the RTS,S vaccine, for children up to 2 years of age. Children are the chosen recipients of the vaccine trial because malaria claims the life of one child every 2 minutes. The goal of the vaccine is to induce high levels of antibodies to both block the sporozoites from entering the liver cells (part of the pathogen's life cycle) and to tag specific infected cells for destruction. However, this vaccine's effectiveness is currently below 50%. Scientists are aiming to increase its effectiveness to above 50%.

Malaria vaccine trial
Read about a new Australian vaccine in clinical trials.

Key concept

By understanding the life cycle of pathogens, scientists hope to eliminate and eradicate disease by targeting the persistence, methods of replication and modes of transmission of the pathogens.

Question set 14.2c

REMEMBERING

1. Describe the generic life cycle of a pathogen.
2. Draw and annotate a diagram showing the life cycle of a *Plasmodium* pathogen and indicate which parts have been targeted as control measures.

UNDERSTANDING

3. Explain why the new vaccine for malaria is being trialled on children.
   a. Describe how the new vaccine impacts the life cycle of *Plasmodium*.
   b. Draw and label the life cycle of another pathogen and indicate where in the life cycle a control measure could be put into place to stop the spread. Explain the reasons for your choice.

Medications

Medications used to treat infectious diseases come in the form of **antimicrobial agents**. The type of antimicrobial depends on the type of organism that is causing the infection: whether the organism is a bacterium, virus, fungus or protist. Antibiotics are medications that treat bacterial infections, antivirals treat viral infections, and antifungals treat fungal infections. There are also some antiprotozoal drugs (such as antimalarials) that treat protistan infections.

Antibiotics

Antibiotics are antimicrobial chemicals that inhibit or destroy bacteria. They target structures or processes only present in bacteria. They are used as a treatment for an infectious disease and can lead to a cure. This results in a decrease in the spread of the disease from the infected host.

Bacteria are prokaryotes, so it has been relatively easy to find and develop antibacterial drugs that have minimal side effects on many other organisms, because eukaryotes have a very different structure. Antibiotic drugs target structural features and metabolic characteristics of prokaryotes that are significantly different from those in eukaryotic cells. Drugs used to treat bacterial diseases can be grouped into categories based on their modes of action. Table 14.1 provides examples of specific antibiotics and their mode of action on bacteria. Without antibiotics, a simple wound or straightforward surgery, such as the removal of an appendix, can become life threatening. Pneumonia would return to being the mass killer it once was.

TABLE 14.1 Mode of action of some antibiotics

EXAMPLES OF ANTIBIOTICS	ACTION ON BACTERIA
Streptomycin	Disrupts protein synthesis
Penicillin	Disrupts cell wall growth in bacteria
Clotrimazole	Disrupts cell membrane permeability

Antibiotics that are classified as penicillins and cephalosporins all interfere with the synthesis of the peptidoglycan layer in prokaryotic cell walls. Because eukaryotes have neither the peptidoglycan components nor the enzymes that synthesise them, these drugs do not affect the host cells. A second class of drugs (including chloramphenicol, the tetracyclines, and erythromycin) binds to prokaryotic ribosomes and inhibits protein synthesis. Prokaryotic ribosomes are structurally different from eukaryotic ribosomes, so these drugs have minimal effect on eukaryotic cells. Nevertheless, some of them may be toxic to some human tissues when they are used in high doses or for prolonged periods of time. Rifampicin is one of the antibiotics frequently used for treating TB. This drug inhibits prokaryotic RNA synthesis.

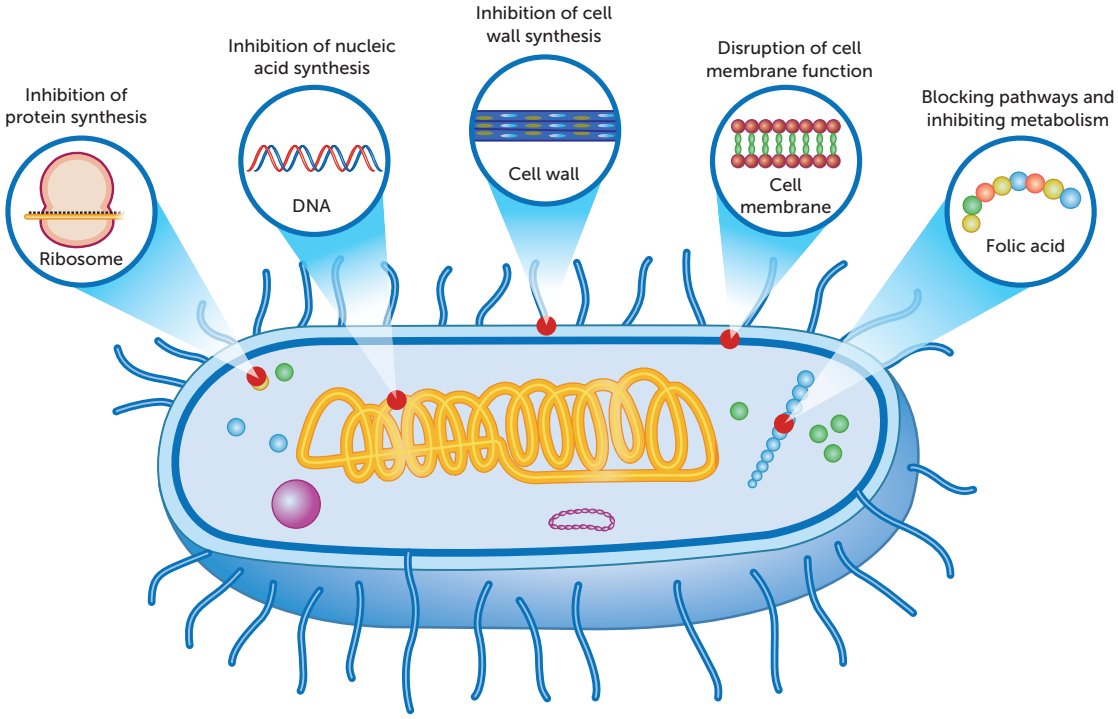

FIGURE 14.9 Modes of antibiotic action

Superbugs that resist antibiotics can evolve in 11 days
View this video to see graphic images about the misuse of antibiotics and an investigation of antibiotic resistance in a giant Petri dish.

Antibiotics have been overused and misused. Misuse occurs when antibiotics have been prescribed and the full course has not been taken. Other times, patients have been prescribed antibiotics as a preventative measure instead of a treatment or they have been prescribed too early, preventing immune systems from building naturally acquired immunity (these are examples of overuse). Resistant bacteria have rapidly evolved. Antibiotics act as a selection agent in the evolution of bacteria, because they give a survival advantage to those bacterial populations with the favourable characteristic of antibiotic resistance. Bacteria that are resistant to antibiotics survive and quickly reproduce, passing on the resistance gene. Over many replications, entire bacterial populations can become resistant to antibiotics as the resistance gene becomes fixed.

Antibiotics that treat TB have become ineffective over time. The *Bacillus thuringiensis* (Bt) bacteria evolved resistance because infected people did not take the complete dose (due to being forgetful or ignorant). Now, a cocktail of antibiotics needs to be taken over a minimum of 6 months for the medication to be effective.

Antivirals

Antivirals are antimicrobial chemicals that inhibit the ability of viruses to replicate. This type of medication only works on viruses, disrupting the life cycle of the virus. If fewer viruses are made, the duration of the disease will be shorter and the spread of the disease will be reduced. Unlike other antimicrobials, they do not deactivate or destroy the microbe. Antivirals treat viral infections by minimising symptoms and infectivity, and shorten the duration of the illness. However, most viral infections will be resolved by natural immune responses.

Most illnesses due to viruses are treated symptomatically until the host's immune system controls and eliminates the pathogen (or the host dies). Antiviral drugs that are used typically target virus-specific enzymes involved in viral nucleic acid synthesis.

Most of the antiviral drugs now available are designed to help combat HIV, herpes viruses (best known for causing cold sores and genital herpes, but actually the cause of a wide range of other diseases, such as chicken pox), the hepatitis B and C viruses (which can cause liver cancer), and influenza A and B viruses. Designing safe and effective antiviral drugs is difficult, because viruses use the host's cells to replicate. This makes it difficult to find targets for the drug that will interfere with the virus without harming the host

organism's cells. Moreover, the major difficulty in developing vaccines and antiviral drugs is related to viral variation. New strains develop rapidly, making previous vaccines obsolete.

Various points in the life cycle of a virus (see Figure 14.10) can be targets for antivirals. Examples of antivirals acting on the various target points include antivirals that:

1. inhibit binding or attachment (known as 'entry blocking' drugs)
2. inhibit entry or penetration by blocking protein channels in the host cell membrane
3. inhibit transcription of the virus by blocking transcription factors to viral DNA
4. prevent the release of the newly assembled viruses from the cell membrane (these have been used to treat a wide range of influenza strains).

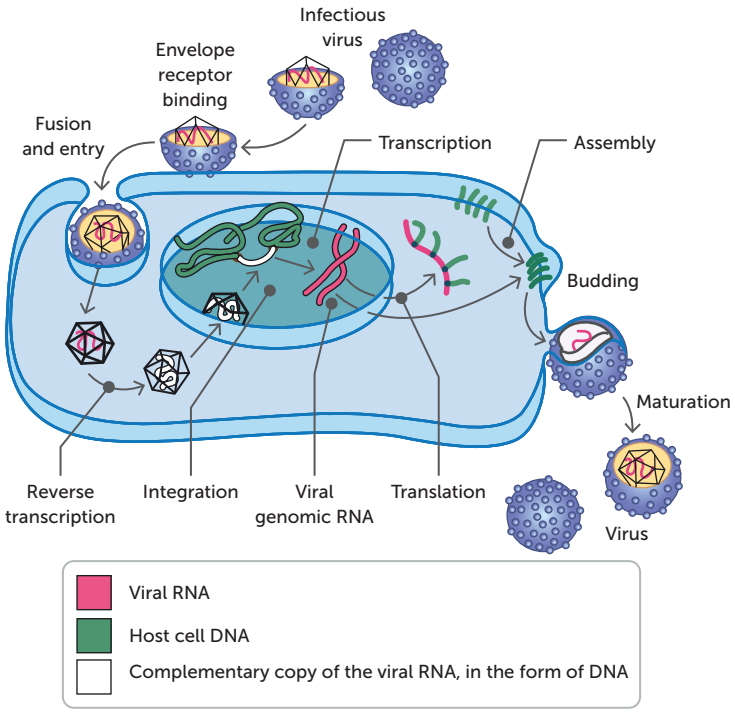

FIGURE 14.10 Life cycle of an RNA virus

Influenza is generally caused by infection with either influenza A or influenza B. Every year there are one or two types of each of influenza A and influenza B viruses circulating. Influenza can be treated by antivirals, for example oseltamivir. WHO recommends vaccination as the primary management against infection and spread. However, after infection, if there is moderate to severe illness, antivirals are recommended by Australian health authorities, but they must be issued as soon as possible after onset of symptoms. Currently, there is no significant evidence of resistance to antivirals in recently circulating strains of influenza A or B.

Question set 14.2d

REMEMBERING

1. Copy this table and list two differences between antibiotics and antivirals.

	ANTIBIOTICS	ANTIVIRALS
Difference 1		
Difference 2		

2. State a reason why it is relatively easy to develop antibacterial drugs that have minimal side effects on humans.

UNDERSTANDING

3. Explain why certain structures on a bacterium have been chosen as targets for the action of antibiotics. Give two examples of these.
4. Explain why new vaccines are made yearly for influenza.

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Physical preventative measures

Prior to the mid-19th century, the transmission of infection was not well understood. In hospitals, surgeons did not wash their hands, and rates of death from post-operative infection were extremely high. In fact, the contamination of a surgeon's clothes with bodily fluids was considered a sign of experience. A British surgeon, Joseph Lister, had read of Louis Pasteur's theory that micro-organisms cause disease, and he hypothesised that preventing their entry may stop disease. Lister experimented with the use of carbolic acid to clean wounds and instruments, as well as for handwashing, as a way of maintaining low levels of micro-organisms and preventing infection. These strategies proved successful in lowering post-operative infection rates, and Lister's practices gained favour with other surgeons. Today, regular handwashing and the use of sterile equipment are considered key elements in effective healthcare. The mouthwash product Listerine® is named after Joseph Lister.

Handwashing is one of several physical preventative measures useful against the spread of disease.

Handwashing

Regular handwashing can prevent individuals from contracting infections, particularly those that are spread by faecal–oral or direct contact routes. On a global scale, handwashing can significantly reduce the **mortality** from certain infections, such as diarrhoeal illnesses.

Handwashing is most effective if warm water and soap or antiseptic handwash is used. An antiseptic handwash contains an antimicrobial substance that inactivates micro-organisms or inhibits their growth. For example, it might contain 60–95 per cent alcohol, which can destroy the cell wall and membrane of bacterial cells and the envelope of viruses (including SARS-CoV-2). Handwashing should include rubbing both sides of hands and in between fingers, finishing with a thorough rinse and dry. This should be done before preparing or eating food and definitely after sneezing, coughing, gardening, toilet duties or looking after sick people. Under certain circumstances (especially after being exposed to an infected host of a disease that spreads via contact or **fomites**), a shower and washing of clothes is recommended. Washing removes or kills pathogens, which prevents the spread of disease. Diseases spread by faecal–oral or direct contact routes are often avoided through handwashing. If hands are contaminated with an infectious agent, they can easily contaminate food, and the agent can then enter a host via the gastrointestinal tract.

Use filtered clean water and food

Water should be treated before being supplied. In many areas of the world, water is unclean and carries waterborne diseases. Pathogens can be carried long distances in water, further increasing the spread of infectious diseases. Sometimes food such as salad ingredients are washed in untreated water, which means infectious agents can be transferred into salads and consumed. Other pathogens are foodborne because the food they occupy is undercooked or out of date when consumed.

Sanitation

Sanitation is the safe disposal of human excreta (faeces and urine). Unfortunately, 2.6 billion people in the world lack adequate sanitation, which leads to the spread of (mainly diarrhoeal) diseases. Many governments build sanitation infrastructure for their communities, but they can only do so if they have the funding. Excrement, especially in urban areas, requires safe removal, transport and treatment. This measure can prevent the oral entry of many infectious diseases, slowing the spread significantly.

Sneeze and cough into elbow

To prevent the spread of infectious disease, the elbow can act as a barrier to any airborne droplets that exit an infected host during a cough or sneeze. SARS-CoV-2, influenza and TB can be transmitted by coughs and sneezes. To avoid transmission, an elbow is used to capture the pathogen. If the hand is used instead of an elbow, the hand is likely to become a fomite or vehicle, and thereby provide an alternative mode of transmission.

Barriers

Barriers to prevent the transmission of disease and therefore the spread include mosquito nets, kitchen gloves and surgical masks. Mosquito nets have been made more effective when sprayed with insecticide. Protection against mosquito-borne diseases such as malaria can last for months, but susceptible hosts need to be diligent in their use of the nets. Infected hosts of *Mycobacterium tuberculosis* can wear a surgical mask. The mask can stop exhaled droplets from being generated or projected. Healthcare workers can wear specialist masks that are designed to protect them from inhaling droplet nuclei, helping protect these individuals from becoming infected with *M. tuberculosis* when in close contact with a person with infectious TB. Masks have been an important management tool in reducing the spread of SARS-CoV-2.

Handwashing saves lives

14.2 APPLICATION

Each year, almost 200 000 hospital-acquired infections occur in Australian hospitals. In 1840s Vienna, Dr Ignaz Semmelweis proved that the unhygienic practices of his medical staff caused septic infections and death in 13% of women after childbirth. Almost two centuries later, hospital staff worldwide are still being told to wash their hands to reduce disease transmission between patients. Handwashing campaigns in Australia have raised hand hygiene compliance in hospitals from less than 50% to more than 75% in recent years.

Questions

1. In which industries do you think it is essential to wash your hands regularly?
2. In public bathrooms, you are often given the choice to dry your hands using a jet dryer or paper towel. Which do you think is more hygienic?
3. How do instant hand sanitizers kill '99.99% of germs without water'?

Key concept

Medications (e.g. antibiotic and antiviral) and physical preventative measures (e.g. handwashing, filtered water, sanitation, sneezing into your elbow and barriers) help to prevent and control the spread of disease.

Management strategies for 10 diseases

TABLE 14.2 Management strategies for 10 diseases

DISEASE	BIOLOGICAL ISSUES RELATED TO MANAGEMENT	STRATEGIES OF PREVENTION AND CONTROL
Influenza	Viruses rapidly develop new strains, making the previous year's vaccinations obsolete. Epidemics are seasonal, usually occurring during winter. Transmission is rapid in crowded areas of high host density. Seasonal influenza is hard to differentiate from other respiratory diseases.	**1** Yearly vaccination – immunisation for yourself and protection for vulnerable people around you. **2** Monitoring and sharing of data (including the virus strain) with state, national and global bodies such as WHO. **3** Handwashing and fomite washing with disinfectant to kill microbes; coughing into elbow or a tissue. **4** Isolation such as staying home from work or school to disable the mode of transmission – close contact via airborne droplets; limiting contact with others. **5** Early treatment with antiviral medication may reduce complications and deaths.

DISEASE	BIOLOGICAL ISSUES RELATED TO MANAGEMENT	STRATEGIES OF PREVENTION AND CONTROL
Ross River virus	A mosquito-borne disease that passes between animals and mosquitoes. There is no vaccine or cure; the disease is usually not fatal but causes discomfort and pain. Viruses are active in WA. In the northern and southern parts of WA, the risk of vector transmission is higher after rainy seasons that leave behind mosquito breeding habitats.	**1** Seek a blood test for a proper diagnosis (it can be misdiagnosed as arthritis). **2** Personal protection such as long sleeves, bed nets, and insect repellent, to deter mosquitoes from blood feeds. A mosquito control program such as spraying with insecticide or removing breeding grounds. **3** Notify the Department of Health so that they can issue public warnings in the areas in which mosquito vectors are active. **4** Medical treatment can reduce joint pain and swelling. Rest. **5** Prevention is best, which involves minimising the risk of being bitten by a mosquito vector. Screen all doors and windows of houses, cover up and apply insect repellent (DEET), particularly at dawn and dusk, when mosquitoes are most active.
Viral diseases of honeybees	There are 24 different honeybee diseases caused by viruses. Many contribute to bee deaths. Deformed wing virus (DWV) represents a major threat worldwide and is transmitted by the varroa mite vector. It is not currently a threat in Australia. Sacbrood is a threat in Australia. The sacbrood virus infects bee larvae after they consume contaminated water, pollen or nectar.	**1** Regularly inspect bee hives for signs of disease. Inspect and quarantine imported bees and bee products. **2** Using DNA sequencing, reverse transcription (RT)-PCR and Southern hybridisation, scientists can detect DWV and sacbrood. **3** Pest management, but this may mean culling the hive. **4** Pre-treatment with pest control such as Apiguard® to kill the vector mite, *Varroa destructor*, or for drone brood culling. (The drone is the male honeybee that produces sperm.) There is no treatment after infection for this viral bee disease. **5** For sacbrood virus, the colony's queen bee should be replaced with one supplied by a reputable breeder (re-queening).
Australian bat lyssavirus (ABL) disease	The vaccine is unique in being successful prior to exposure, but also in between entry of pathogen and onset of symptoms. After the onset of symptoms, mortality is usually 100%. The virus is in the same family as rabies.	**1** Vaccinate prior to exposure, especially if travelling to infected areas. The vaccine is used as both prevention and treatment. **2** Educate the public that fast treatment is required. **3** Wash the wound with disinfectant. If not washed immediately, there is no effective treatment or cure once symptoms are established.
	Less than 1% of bats may be infected. Sick or injured bats are more likely to be infected and more likely to be handled. Risk of spread is low, but the risk of infection being fatal is high. Habitat loss acts as pressure on populations, which can influence density and cause a higher rate of transmission between bats.	**4** Do not touch bats. If they are injured, contact the Department of Biodiversity, Conservation and Attractions (WA). Only experienced, vaccinated people should handle bats and it should be done while wearing puncture-resistant gloves.

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DISEASE	BIOLOGICAL ISSUES RELATED TO MANAGEMENT	STRATEGIES OF PREVENTION AND CONTROL
TB	Easily spreads globally because it can be spread by infected people when they travel. *M. tuberculosis* is transmitted only through air containing microdroplets of TB organisms. It is not transmitted by touching surfaces such as bed linen, toilet seats, shaking hands etc. Depending on the environment, tubercle bacilli can remain suspended in the air for prolonged periods, and air currents can carry them throughout a room or building. Multidrug- and extreme multidrug-resistant bacteria have evolved. Hosts with active and latent TB are advised to be treated to avoid transmission. This is because hosts with latent TB can develop contagious active TB at an unpredictable time. Usually, it is not until infected hosts experience a prolonged cough (2–3 weeks) that they get tested. The disease may have spread. About one-third of the world have TB, many without knowing it.	**1** Respiratory protective equipment: patients can wear a surgical mask that stops exhaled droplets from being generated; healthcare workers can wear specialist masks that are designed to protect them from inhaling droplet nuclei. **2** Infected people can be isolated, particularly in hospitals, in respiratory isolation rooms, so that air from the room does not circulate into other rooms. **3** Cough into tissues. **4** Educate and counsel family members about minimising the risk of transmission. **5** Chest X-rays or sputum tests for diagnosis of active TB. Free X-rays are performed in the Western Australian Tuberculosis Control Program, at the Anita Clayton Centre in Perth. **6** Treated with a mix of antibiotics for a minimum of 6 months, but this is only effective if it is taken without interruption; reducing the development of resistance. In WA, anti-TB medication is free of charge, unlike in many areas of the world. **7** Government healthcare workers have infection control guidelines for prompt detection of infectious patients, airborne precautions and treatment.
Tetanus	Is infectious but not contagious. Spores of *Clostridium tetani* are found everywhere in the environment and therefore cannot be eradicated. The neurotoxin causes the infected host harm, but not the bacteria.	**1** Vaccinate with a modified toxin to stimulate immunity. **2** Three booster vaccinations should be administered during adolescence. Immunisation is then stimulated to last throughout much of adulthood. Boosters are required, however, because memory cells decline over time. **3** Wash and disinfect puncture wounds. See a doctor at once.

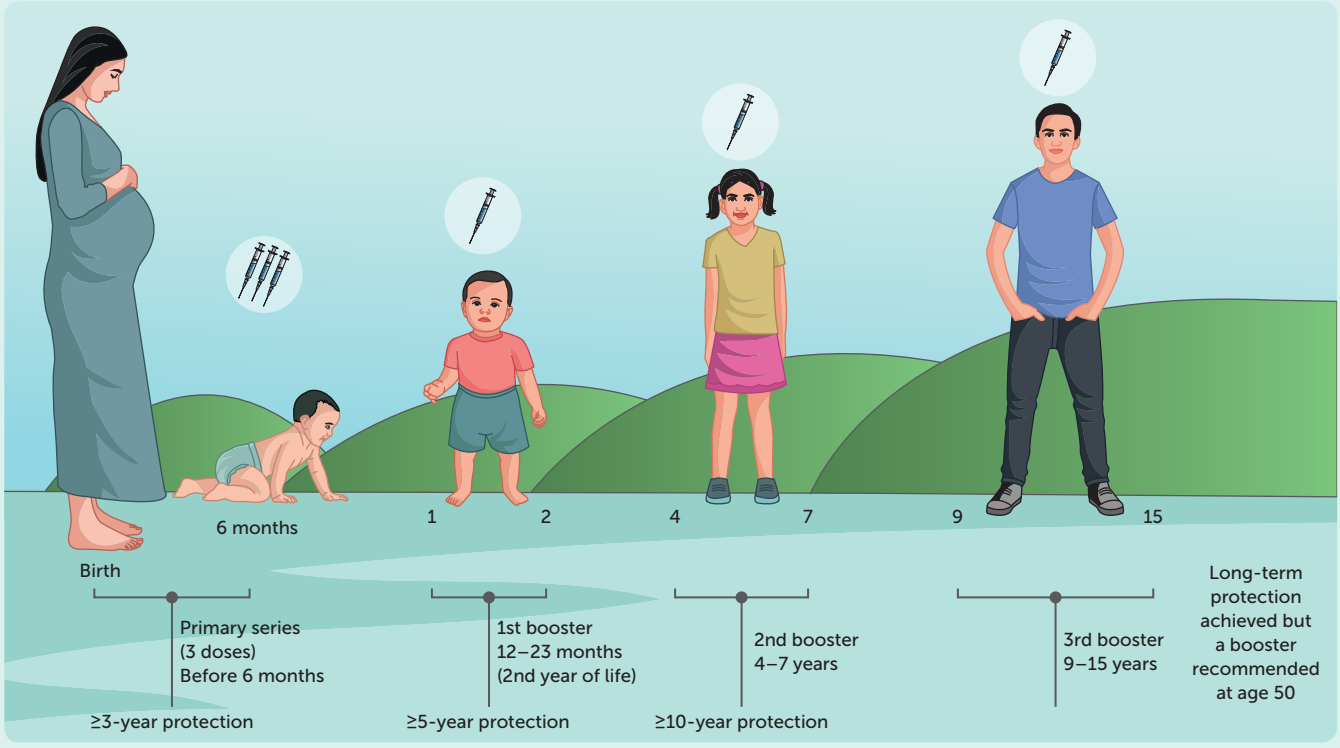

FIGURE 14.11 Tetanus vaccination for long-term protection

DISEASE	BIOLOGICAL ISSUES RELATED TO MANAGEMENT	STRATEGIES OF PREVENTION AND CONTROL
Crown gall	Invasion of spores requires a root wound. Spores are transported via rain and runoff. Inside vascular tissue, bacteria reproduce via binary fission. Bacteria transfer a plasmid gene into the host cell genome so the host plant cells will perform uncontrolled cell division to form galls. There is no cure. It commonly affects roses and fruit trees.	**1** For nursery plantings, do not use soil in which crown gall infection of plants has occurred. Soil used in the nursery should be treated to eradicate crown gall bacteria. **2** Eliminate any plants with galls or suspicious swellings at the graft union or near the soil level. Dig up the plant and soil immediately around the roots and dispose of it carefully, such as by burning. **3** Treat pruning, budding and grafting tools with a disinfectant. **4** Wash down tyres and boots before leaving a contaminated area to prevent spread. **5** Careful surgery to remove galls from trees. After surgically removing the gall, apply heat to dry and sterilise the area around the gall. Monitoring regrowth is required every few months. This is a costly and labour-intensive strategy. **6** Re-direct runoff, especially during rainy seasons, because spores can spread in water.
Chytridiomycosis (amphibian chytrid fungus disease)	The fungus is temperature sensitive. Chytridiomycosis has been found in all Australian states and in the Australian Capital Territory, but not in the Northern Territory. Currently, it appears to be confined to the relatively cool, wet areas of Australia. Australian upland frog populations have suffered the greatest number of declines and extinctions, leading to the suggestion that environmental stress, perhaps from climate change or increased exposure to ultraviolet radiation, may be reducing resistance to infection. Vaccines have been tested on amphibians, but no significant differences in mortality or virulence have been observed.	**1** Control efforts should be aimed at protecting uninfected areas. **2** Detect new outbreaks in currently uninfected populations or locations of unknown disease status. Establish restricted areas and control areas for which quarantines and movement restrictions are applied. Identify infected and non-infected areas. **3** Identify and prioritise Australian frog species at risk of extinction. Species-specific research is needed due to different ecological requirements, as well as captive insurance colonies to avoid extinctions; this means maintaining at-risk species in captivity to be reintroduced at a later date. **4** Monitor impacts on frogs, especially populations who recover naturally. Analyse the different strains of the fungus for different virulence factors and for mapping their locations. **5** Implement hygiene protocols common across all states of Australia: protocols for bushwalking, fishing, four-wheel driving and bike-riding. Disinfecting vehicles can prevent the spread into uninfected areas. **6** Develop a central information storage site at which government, stakeholders and researchers can upload and access data. **7** There is no cure.

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DISEASE	BIOLOGICAL ISSUES RELATED TO MANAGEMENT	STRATEGIES OF PREVENTION AND CONTROL
Malaria	Breeding sites can form, due to rainfall or flooding, in unpredictable areas. It is hard to eliminate the sites as quickly as they form. The distribution of breeding sites enables the distribution of the vector, which in turn influences the distribution of the disease. Conversely, the spread is subject to the distribution of specific species of mosquito vectors (near the equator, where there are warm temperatures and regular rainfall). Strains of *Anopheles* mosquitoes have evolved resistance to insecticides and anti-malarial drugs. Malaria, a protozoan disease, was successfully treated for many years with chloroquine. In recent decades, *Plasmodium* species that are resistant to this drug have appeared and spread to areas where malaria is a common threat. In those areas, a combination of the drugs sulfonamide and pyrimethamine is frequently used to treat the disease.	**1** Use chemical insecticides to kill mosquito vectors and their larvae. **2** Eliminate stagnant (still) water to remove breeding sites of mosquito vectors and reduce vector populations. **3** Avoid being outdoors when mosquitoes are most active, that is, at dawn and dusk; use bed nets at night and protective clothes during the day as barriers to stop transmission, or repellents to deter the mosquito from taking a blood feed; install insecticide-coated mosquito nets and screens on windows and doors. **4** If proven safe, introduce a biological control such as mosquito fish to feed on mosquito larvae, but evidence for the effectiveness of this is limited. **5** Take anti-malaria drugs to reduce the number of people infected with malaria, reducing the spread. There is no vaccine currently available in Australia, although new vaccines are in development.
Phytophthora	Spores do not need a wound for entry. They germinate upon contact with moist, nutritious media, then hyphae emerge from the spore. The hyphae penetrate epidermal cells on the roots. The invasion can spread to vascular tissue, absorbing nutrients and blocking plant host transport of water and nutrients. After symptoms appear, death follows rapidly.	**1** Quarantine strategies consist of restricting access to infected areas to limit the transport of spores out of the area and into uninfected areas, especially during rainy seasons, because contaminated soil or mud can more easily be picked up by vehicles such as boots. **2** Application of phosphite, a biodegradable fungicide, increases resistance to infection. Plants can be injected and protected for up to 5 years. The phosphite is supplied agronomically, either by injection or by foliar spray, but when trees are sprayed, the length of protection is reduced to 2 years. It is safe, inexpensive and has low toxicity to animals. However, decreasing sensitivity of *Phytophthora* to phosphite has been documented. In orchards in both South Africa and Australia, the use of phosphorous acid sprays has led to decreased sensitivity to phosphite. This may mean effective injections will require higher doses. In 2019, the Western Australian Government advised that spraying should be used in conjunction with the 'Pegg Wheel' management strategy for managing phytophthora dieback in avocados (named after its designer, Ken Pegg) (Figure 14.12, page 490). **3** Sterilise or wash down grafting tools, clothing, footwear, equipment and cars before and after use to prevent carrying pathogen spores from infected area to uninfected area; do not carry plants from infected areas into susceptible areas to reduce the risk of spread. **4** Destroy all infected trees to reduce the risk of spread because there is no cure. **5** Schedule work in infected areas during dry seasons because the pathogen spreads more easily in wet and muddy conditions. Redirect runoff during rainy seasons to prevent the spread in disease-free areas.

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FIGURE 14.12 The Pegg Wheel Management Strategy for managing phytophthora dieback, particularly used for avocado trees

CASE STUDY

Managing the spread of disease: coronavirus disease (COVID-19)

WHO monitors, communicates and coordinates health initiatives. They include over 7000 representatives from a diverse array of nations, working collaboratively with global partners such as the UN, research institutions and medical professionals. On 31 December 2019, COVID-19 was first reported to them from Wuhan, China. It was a disease that could cause pneumonia and, in some cases, death. Spread of the disease was swift. Within 3 months, on 11 March 2020, WHO classified the disease as a pandemic. WHO collected and analysed data from its global partners and publicly communicated information about the spread via its 'COVID-19 situation dashboard'.

WHO's pandemic management strategies included helping countries obtain supplies of personal protective equipment (PPE) and providing guidelines. The goal was to equip all governments and health workers to manage **cases** and reduce the spread. Knowledge of the biology and ecology of the pathogen and its disease was essential for effective management planning.

Professor John Mackenzie is an Australian who is working with WHO as a consultant on COVID-19. He was a Professor of Tropical Infectious Diseases at Curtin University, WA, and has spent much of his career analysing global aspects of infectious disease management (surveillance and response), particularly of emerging zoonotic diseases such as avian influenza, Australian bat lyssavirus and Ross River virus disease.

Structure and source of the virus

The virus responsible for COVID-19, SARS-CoV-2, is a novel (new) zoonotic RNA virus.

From phylogenetic tree analyses of the available full genome sequences, bats appear to be the reservoir of the SARS-CoV-2 virus, with a potential intermediate host(s). Information on animals that were in the marketplace in

Huanan, the place thought to be the origin of the disease, has been recorded, along with the post-market destinations, in case those animals might be a source of further, potentially zoonotic, outbreaks.

Signs, symptoms, disease progression and severity

Disease presentation can range from no symptoms (asymptomatic) to severe pneumonia and death. Typical signs and symptoms include (in order of highest to lowest incidence): fever, dry cough, sore throat, fatigue, sputum production, shortness of breath, headache, muscle aches, chills, nausea or vomiting, nasal congestion and diarrhoea. The mean incubation period (time period between exposure to the virus and observable symptoms) is 5–6 days, with a range of 1–14 days. Most people infected with the SARS-CoV-2 virus have mild disease and recover, but 13.8% have severe disease. Approximately 80% of humans infected with the SARS-CoV-2 virus have mild symptoms, especially children and young adults, and recover, but around 20% have severe disease. Individuals at the highest risk for severe disease and death include people aged over 60 and those with underlying conditions, such as cardiovascular disease.

Transmission routes

SARS-CoV-2 is transmitted via droplets and fomites during close unprotected direct contact between an infected host and a susceptible host. Airborne spread may be possible for COVID-19, but it has not been observed to any great extent to date. Human-to-human transmission is largely occurring in families. SARS-CoV-2 is transmitted directly via airborne droplets and fomites during close contact (within around 1.5 m between an infected host and a susceptible host). When an infected host coughs or sneezes, droplets containing the virus may be inhaled by a susceptible host. Indirect transmission can occur when a susceptible host touches a contaminated surface, called a fomite, and then touches their eyes, ears or mouth. This is because many droplets fall on nearby surfaces and objects, such as tables or phones. The virus can persist for a few hours and up to a few days depending on factors such as temperature and humidity. Due to the ease with which this pathogen spreads and persists, organisations such as WHO and governments require management plans that are suited to this specific, highly contagious disease. As we do not currently have any therapeutic medicine or vaccine for the new coronavirus, much of the management is undertaken by public health authorities.

Management plans

1. Management of cases: Health workers require a plan for assessing cases as mild, moderate or severe, and treating them accordingly. Respiratory support may be necessary. Establishing fever clinics, increasing emergency wards, and even opening new temporary hospitals are likely to be required. Assistance may be needed for at-risk people. Special plans for palliative care, pregnant women and ethical considerations are needed. Decisions about medical care need to be based on the premise that every person is equal, rather than on discriminatory factors such as age or sex.
2. Testing and **contact tracing:** need to be efficient to reduce spread. The PCR test uses a method called reverse-transcription polymerase chain reaction. If the virus is present in a sample, copies are produced of the virus' genetic code, which is in the form of RNA, and the results can be returned the same day. If a patient's test result is positive, they need to be quarantined. In Australia, positive cases are reported to the Department of Health and Human Services.
3. Stopping or slowing transmission via education and physical preventative measures: these measures include social distancing, quarantine, isolation, stopping mass gatherings such as sporting events, closing schools and universities, and (where possible) having people work at home. Frequent handwashing, regular disinfecting of potential fomites, wearing a mask and always covering mouth and nose when sneezing or coughing are important.

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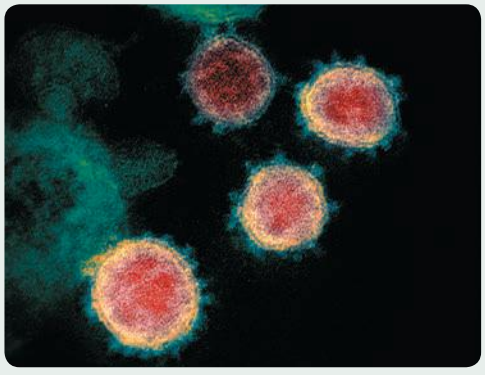
Coronavirus outbreak: How the COVID-19 vaccine is being made
Watch the video and read the content to find out about the stages involved in creating a vaccine.

4 Border biosecurity: National border security involves travel restrictions, international air arrival passenger caps and two weeks' quarantine on arrival at a designated facility. During a pandemic, flights are limited, and only applicants who are approved for exemption may travel. In WA, strict state border security has been enforced during the pandemic. People have not been allowed to enter WA without a special exemption, and eligible people have been subject to mandatory, self-funded quarantine.

5 Vaccine research: Vaccine research is being carried out by a number of universities, health research institutes and companies around the world. An important vaccine research group is based at the University of Queensland. They are being funded by international agencies to develop a vaccine to SARS-CoV-2. They have developed and patented new 'molecular clamp' technology, which it is hoped will make it possible to rapidly develop an effective vaccine. Once a vaccine is created, it will need to be quickly scaled up and distributed.



Alamy Stock Photo/ American Photo Archive

FIGURE 14.13 An electron micrograph of SARS-CoV-2, the virus that causes COVID-19

Emerging respiratory viruses
Find out more about methods of detection, prevention, response and control for viruses such as SARS-CoV-2.

Vaccine development

In the initial steps of developing a vaccine for COVID-19, the genome of the virus was sequenced and the structure of the virus studied. The genome is 29 878 base pairs long. It was sequenced several times to monitor whether the virus was mutating and evolving, which it was. Scientists involved in the vaccine development need to include this mutation factor in their planning.

The virus was grown in labs around the world, including the Peter Doherty Institute for Infection and Immunity in Melbourne, to study it and to have samples ready for vaccine testing. Animal models at the CSIRO centre, also in Melbourne, have been used for testing potential vaccines. Ferrets were chosen as an animal model because they are susceptible to the SARS-CoV-2 virus and develop symptoms similar to humans. After a vaccine is found to be safe and effective in animal trials, human trials are to be conducted.

Currently there are three types of virus vaccines: subunits (that contain parts of a virus), live attenuated vaccines (that contain a weakened form of a virus) and inactivated viruses (that contain 'dead' viruses that cannot replicate). Scientists at the University of Queensland are applying a new vaccine technology developed in Norway that uses features of the proteins found on the surface of the virus.

The coronavirus invades a human host cell to replicate, using 'spike' proteins found on the virus' outer surface, which are coiled up like tiny springs. The structure of the spike proteins enables the outer virus coat or 'envelope' to merge with the cell cytoplasmic membrane, allowing the virus core containing the viral RNA genome (along with protective proteins and enzymes) to enter the cell cytoplasm.

Our immune system responds by recognising the antigens (spike proteins) of the virus and making specific antibodies to kill it. The new molecular clamp technology at the University of Queensland is being used for developing a vaccine based on the spike proteins. The function of the molecular clamp is to fix the protein spike in its characteristic coiled shape, so that the host's antibodies can recognise it.

It has been determined that the virus is mutating. Scientists are now trying to discover whether it is like the flu virus, which mutates quickly and therefore needs a new vaccine every year to account for the mutational changes, or whether it is like the mumps, so that only one vaccine will be required.

Vaccine development
Here's why a vaccine can take more than 18 months to be developed.

Fairfax Photos/Scott McNaughton

FIGURE 14.14 Professor George Lovrecz and Mylinh La investigating a potential COVID-19 vaccine in Melbourne's CSIRO facility.

Questions

1. List the major facets of a management plan for a disease such as COVID-19.
2. Describe how social distancing slows transmission.
3. Describe some physical preventative strategies you can use to prevent infection.
4. Explain why a vaccine has been difficult to create.

SCIENTIFIC LITERACY

This mite be the bees' worst enemy

Bees, those small insects that collect nectar and pollen and make honey and wax, are in precipitous decline: populations in the US and Britain, for example, have halved over the past 25 years.

Their biggest threat is from the evil-sounding *Varroa destructor*, an oval-shaped, reddish-brown mite that sucks the blood from bees and transmits virulent diseases, such as deformed-wing virus. The pinhead-sized bloodsuckers have decimated bee populations worldwide, including in neighbouring New Zealand and Papua New Guinea, but have not arrived yet in Australia.

'If they enter this country, the mites will completely wipe out our wild honeybees, which means crop growers will lose their largest and free source of pollination, worth more than $1 billion a year,' says bee pathologist Denis Anderson of CSIRO Ecosystem Sciences in Canberra.

The mites will also reduce the number of managed honeybee colonies, he explains. 'This means keepers will pay more for scarce paid pollination services – costs that [will] flow through to consumers.' In addition, most of Australia's horticultural and agricultural crops, worth billions of dollars, rely on bees for pollination.

Australia's national port surveillance program, although currently inadequate to deal with the threat, is being strengthened. Surveillance for honeybees and bee pests and parasites that are exotic to Australia forms part of the National Sentinel Hive Program, which is coordinated by Plant Health Australia.

The program, established in 2000, has been growing steadily, Dr Anderson says. 'It operates on the premise that most of the important exotic pests and parasites will enter Australia on live honeybees from another country – particularly through bee swarms arriving on vessels at our sea ports,' he explains. 'If a swarm was carrying exotic parasites, such as the *Varroa* mite, those parasites would spread to colonies near the port, and then on to colonies further away.'

The National Sentinel Hive Program places special hives at sea ports around Australia and monitors them every 2 months for signs of exotic pests and parasites that may have arrived in a bee swarm from overseas.

The program's success depends on how many hives are at each port and the number of ports targeted. 'The more of each, the higher the chance of success,' he says. 'At present, only a few hives are based at a few strategic ports – just three hives cover Melbourne and Geelong, for example – and there are not enough funds to expand the current program.'

This is why the state government has set up Bee Force, a pilot project to improve Victoria's capacity to detect incursions of exotic bee pests. Now being trialled in Melbourne and Geelong, the project involves local amateur beekeepers who run sentinel hives.

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Shutterstock.com/ Igor Chus

FIGURE 14.15 The varroa mite: **a** the mite is small, shiny and reddish-brown; **b** extreme close-up of the mite on beehive cells containing bee eggs and larvae

'This encourages community involvement and expands the number of sentinel hives that can be used for surveillance, thus keeping costs to manageable levels,' Dr Anderson says. 'If the trial Bee Force program proves successful, it could be extended to other port areas.'

Victoria's Department of Environment and Primary Industries has trained a honeybee quarantine response team of 90 beekeepers, including hobby and commercial beekeepers, who may be called on to assist in an emergency.

The early detection of the *Varroa* mite, combined with effective surveillance, it is argued, may increase the chance of eradicating the parasite once it enters the country. This has never been achieved before.

'Since it switched host (from the Asian honeybee to European honeybee), the mite has spread throughout the world,' Dr Anderson says. 'Let's ensure we do everything possible to keep the parasite out of Australia for as long as we possibly can.'

Questions

1. List at least three industries that could be affected were the varroa mite to spread into Australia.
2. Australia is now the only country in the world with a honey industry that is free of the varroa mite. Spread to New Zealand and Hawaii only occurred relatively recently.
   - **a** What characteristic of these three countries allowed them to remain mite-free for so long?
   - **b** Explain how strategies to prevent the introduction of *Varroa destructor* into Australia utilise this characteristic.
3. Brainstorm some other strategies that could be used to prevent the introduction of *Varroa destructor* into Australia.

Question set 14.2e

REMEMBERING

1. Describe two different types of physical barrier that prevent the spread of disease.
2. Describe two methods of removing or killing pathogens.

UNDERSTANDING

3. Apply your new knowledge of physical preventative measures to a disease other than TB or tetanus, but one you studied during this course.
4. Evaluate two measures you chose for Question 3, describing at least one benefit and one issue.

APPLYING

5. Construct a mind map of the 10 diseases in Table 14.2, including their issues and unique management strategies.

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14.3 MONITORING DISEASE ACTIVITY

In order to define and control disease outbreaks, public health authorities need to know when and where particular infections are occurring. In Australia, the number of cases of a particular disease is monitored by health authorities in each state. On a global level, the monitoring of diseases is conducted by WHO, the organisation that coordinates global responses to outbreaks that pose widespread threats.

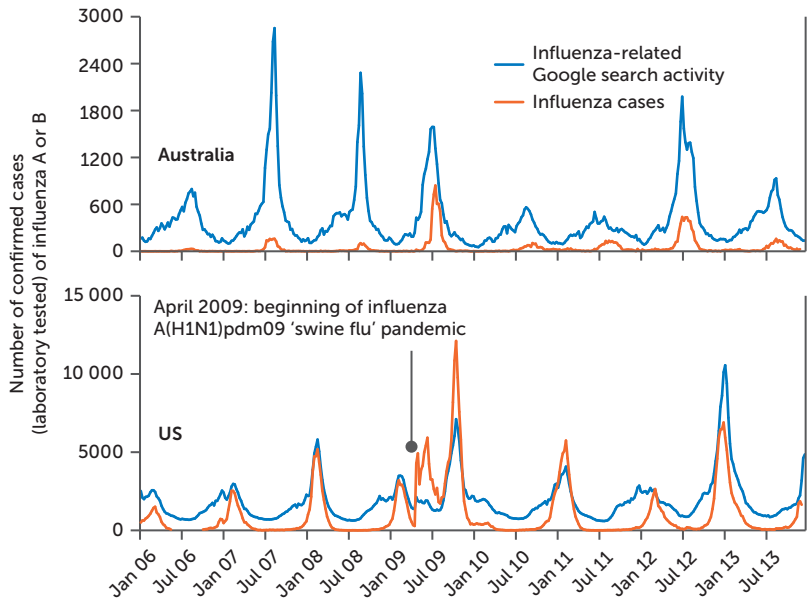
Notifiable diseases in Australia
Visit the Department of Health's website to view the current list of notifiable diseases in Australia.

National surveillance

A widely used method of monitoring disease activity is through the notification of public authorities when individuals are diagnosed. In Australia, the list of notifiable diseases contains a diverse mix of more than 70 conditions, including chickenpox, syphilis, rabies and influenza. A doctor who diagnoses one of these conditions must report the case to the relevant state health authority. Outbreaks or cases of unusual diseases can then be investigated.

There is a number of limitations to data collected in this way. Not all patients who are infected with a disease will seek healthcare, and of those who do, not all will receive a diagnosis. Infections can also be under-reported, and this may be more of a problem in certain populations or with certain diseases. Together, this means that reported data are likely to be lower than the actual number of cases. There can also be delays between the onset of symptoms, diagnosis and reporting, which can limit the ability of public health authorities to respond quickly to epidemics.

In recent years, researchers have been exploring alternative ways of conducting disease surveillance. The widespread use of the Internet and social media provides a novel data source from which information about the frequency of different diseases can be extracted. Data from Facebook, Twitter and mobile phones have been used to monitor disease activity. Google has developed a program that tracks how frequently people use the search engine to look up influenza-like illnesses. Figure 14.16 compares data obtained by this method with traditional reporting data. You can see that the spikes in Google search activity correspond with the peak influenza season.



Google Flu Trends
Visit Google Flu Trends for up-to-date data on influenza-related searches.

FIGURE 14.16 Patterns of influenza infection and Google search activity related to flu-like illness in Australia and the US

These digital disease surveillance mechanisms have the advantage of providing information to public health authorities in real time. However, the quality of the data is limited by the effectiveness of the algorithms in determining whether or not a tweet or search is actually about an illness. Furthermore, high Internet activity about an illness does not always correspond to high disease activity, as it can be skewed by other events, such as the illness of a celebrity.

Due to its limitations, digital disease surveillance is not likely to replace traditional reporting methods. It does, however, provide epidemiologists with an additional tool that complements these methods.

Managing an outbreak

Epidemiologists take a number of steps to investigate a new disease outbreak (Figure 14.17). Similar steps apply whether the outbreak is a small, localised occurrence or a pandemic. When an outbreak is suspected, the first step is to confirm that the reported cases do, in fact, meet the definition of an outbreak. This involves confirming the number and diagnosis of known cases and comparing this with background levels of the disease.

Once this has been done, investigators can formulate a **case definition**, indicating which cases are considered to be part of the outbreak. Case definitions include not only the type of illness but also the place and time. Such definitions can change and be refined as the investigation progresses and investigators understand more about the disease. The next few steps (finding cases, gathering information and developing hypotheses) are easiest if the mechanism of transmission of a disease is already known. When the mechanism is not known, investigators have to consider a wider range of potential cases and disease sources.

Investigators, then, need to find people affected by the outbreak. Not all of those who are ill will have sought medical care, or been reported to investigators. As such, disease surveillance (or passive case finding) generally does not locate all individuals affected. Investigators in disease outbreaks perform active case finding, in which they try to track down infected individuals. An important component of this is contact tracing, whereby people who may have infected, or been infected by, known cases are tracked down. The type of contacts sought will vary with the mechanism of transmission. For example, if the disease is sexually transmitted, only sexual contacts of the infected individual will be contacted by investigators. On the other hand, for an airborne disease such as TB, investigators will contact all people who have been in close proximity with the case. In some cases, investigators may focus on people who have been exposed to the same potential sources, even if they didn't have direct contact with an infected individual. In the measles example in the weblink, it was concluded that the infection of at least 20 people could be traced back to one man who visited Perth from New Zealand, based on efforts made to contact people with symptoms who might have met him. As part of the case findings, investigators may be able to identify the **index case**, or the case that started the outbreak. In the Perth measles outbreak, it appears that the visiting New Zealander was a super-spreader who was identified as **patient zero**.

Investigators will then gather information from cases. Initially, this will involve in-depth interviews to explore any potential sources of infection. These interviews will include asking about usual activities, sick contacts, recent meals and travel. The aim of these interviews is to generate a hypothesis about how the outbreak is spreading.

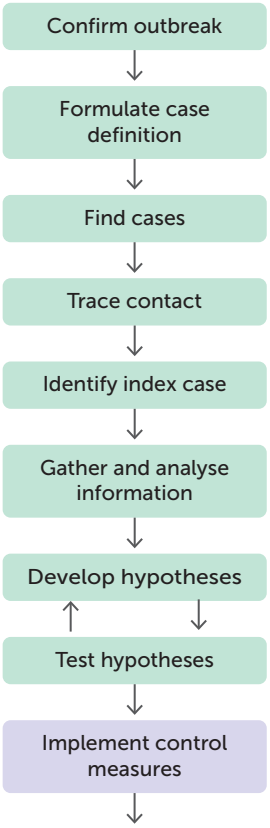


FIGURE 14.17 The steps involved in investigating a disease outbreak. Communicating findings and implementing control measures usually happen throughout the investigation.

Tackling the WA measles outbreak
Read the article to find out how quickly and easily an infectious disease can spread.

Solving the outbreak

Once a hypothesis has been generated, the investigators can search for evidence to support or refute that hypothesis. This evidence might include further interviewing of cases, site inspections, and environmental sampling (such as testing water or food for pathogens). In some cases, sufficient data may have already been collected, and testing the hypothesis involves analysing that data.

An outbreak investigation is an important aspect of controlling a disease outbreak. The investigation involves a series of steps that aim to determine what has caused the outbreak. The final steps of an outbreak investigation are to implement measures to control the spread and to communicate the findings. In practice, both of these steps may take place while other steps are being undertaken.

Predicting the spread of disease

Mathematical models that can predict the spread of disease are important tools in the control of outbreaks. Such models can be used to explore the likely effects of newly emerging pathogens and changes in environmental conditions. They can also be used to design models and predict the chance that a disease will invade particular countries, the expected number of cases within a particular time frame and the effects of potential public health interventions. Contemporary models project how the disease will progress and simulate the effects of possible interventions. Such models are used to inform public health interventions, such as mass vaccination programs.

Supercomputing has increased processing capacity, and data storage has enabled models to increase in their complexity, with new variables being examined and new relationships found, such as the relationships between epidemic frequency and location, and factors such as population size, environmental change, persistence and antibiotic resistance.

In order to make these predictions, mathematical models include several assumptions about the way that different variables behave. The accuracy of these models is dependent on these assumptions being met. You have already seen how a large number of factors can impact on disease transmission. In order for a model to have good predictive ability, the design of the model needs to reflect this complexity. The use of mathematical models to predict disease spread involves close collaboration between mathematicians and biologists.

For this information to be of value, the model must be a sufficiently accurate representation of reality in order to provide useful outputs. All models have a trade-off between complexity and accuracy, so it is important to assess which approach is most appropriate for each individual situation. How reliable the predictions are depends on how robust the data are, how quickly they are accessed and how accurate the assumptions are. If the results of a prediction are not reproducible, then their reliability is questionable. Predictions about emerging diseases have less reliability due to the lack of data.

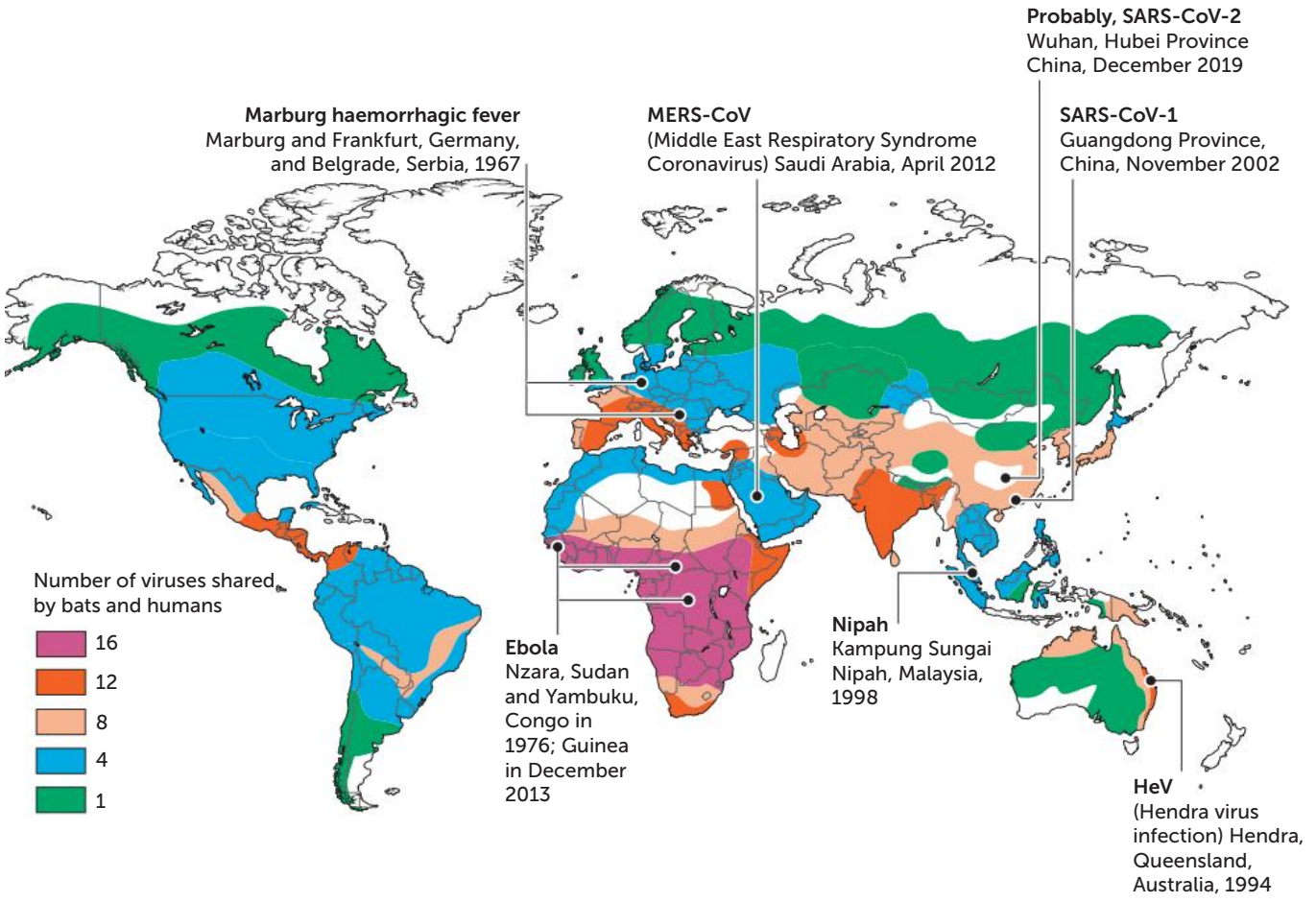
Data modelling underpins public safety
Watch the video and read about the findings of the model here.

Educating for public safety

A disease modelling expert at the University of Western Australia has modelled a potential outbreak of COVID-19 in Perth and warned that community education for a future outbreak should be a priority to help people cope with lock-down and social-distancing measures. The modelling has also shown how to effectively wind back social distancing without causing another outbreak. The study indicated that in this example 14 weeks of social distancing was required to significantly reduce the chance of an outbreak reoccurring.

Key concept

Monitoring of pathogens and the outbreak of disease occurs at the national and international level in order to effectively manage, predict spread and implement disease control measures.



Sources: World Health Organization (WHO),Centers for Disease Control and Prevention.

FIGURE 14.18 Map indicating the locations of bat-to-human transmission of zoonotic diseases. This data can be used to predict possible locations of future emerging zoonotic disease.

Question set 14.3

REMEMBERING

1. Outline the steps in performing an outbreak investigation.
2. Explain what a notifiable disease is, giving an example.

UNDERSTANDING

3. Explain how mathematical modelling can aid epidemiologists in controlling disease spread.

CHAPTER 14 ACTIVITIES AND INVESTIGATIONS

Modelling disease spread

14.1 ACTIVITY

Models are tools employed by epidemiologists to predict the impact of different factors on disease spread. The disease lab simulator allows you to modify various disease characteristics (infectivity, mortality rate and duration of infection), population density and vaccination status, and observe their impact on the spread of disease.

Aim

To explore the impact of several variables on disease transmission.

What to do

1. Access the weblink and perform your own investigation. (The first page of the the weblink provides a link to the simulator and outlines how to use it.) Write up your investigation using the standard scientific report format, from Aim to Conclusion (refer to Chapter 1, if you are unsure how to write up your report).
2. Using what you have learned in this activity, discuss how the risk of pandemics spreading to Australia is different now compared with 100 years ago, and identify the pathogens, hosts and environmental factors that contribute to this risk.

Disease lab
Use the interactive lab simulator to investigate the spread of disease.

Outbreak management in Australia

14.2 ACTIVITY

Background

Honeybees are vital for pollination of our crops and flowers in Australia. Globally, honeybees are suffering from various infectious diseases, many of which are caused by viruses. Fortunately, several of these pathogens have been prevented from entering Australia due to our geographically isolated location and strict biosecurity. Read page 438 to find out about the epidemiology and pathology involved in the deformed wing virus (DWV) disease. Epidemiologists look for typical clinical signs of DWV: shrunken, crumpled wings, decreased body size, and discolouration. However, adult honeybees with high levels of the virus may not show clinical signs. Finding the source of the disease would be difficult.

Aim

To learn about the control of disease outbreaks in Australia through the examination of a recent case

You will need

- A computer with Internet access
- A large sheet of poster paper
- Markers and pens

What to do

1. Perform an Internet search about the DWV disease and its outbreak, aiming to find the following information:
   - characteristics of the disease (for example, the type of pathogen, symptoms, mortality, incubation period and mode of transmission)
   - size and impact of the outbreak
   - outcomes of the epidemiological investigation (for example, was a source found?)
   - control measures used by public health authorities.

Honeybee report
Find out about a major threat to Australia's honeybee and crop pollination industries.

→

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2 Summarise the information on the poster paper. You could use a flow chart or timeline to show how events unfolded.
3 Using your poster, explain to a classmate what you have found.
4 What sources of information did you choose to use? Explain why you chose these sources and how you know that they are reliable.

What did you discover?

1 Outline the process of investigating an outbreak, using your case as an example.
2 Explain, using your case as an example, how the mode of transmission of a disease can direct an outbreak investigation.
3 If an outbreak of DWV disease occurred in Australia, what steps do you think epidemiologists would take to ensure it was correctly identified, the source found and spread controlled?

14.1 INVESTIGATION

The efficacy of alcohol-based antisepsis

Background

Alcohol-based hand rubs are widely used in hospitals as an alternative to frequent handwashing with soap and water. To use the hand rub, you simply squirt a small amount into the palm of your hand and rub your hands together so that the liquid covers your hands. The alcohol rapidly evaporates, leaving your hands dry. In this experiment, you will compare the efficacy of alcohol-based hand rubs with that of traditional handwashing.

How to handrub? How to handwash? WHO guidelines for handwashing and using alcohol-based hand rubs

Aim

To determine whether alcohol-based hand rubs or handwashing with soap and water is more effective in reducing bacterial load on hands

Materials

- Alcohol-based hand rub
- Liquid soap (not antibacterial handwash)
- Sink with water
- Paper towel
- Sterile agar plates (two per student)
- Clear tape or Parafilm
- Hand Hygiene Australia or WHO guidelines for proper handwashing and use of hand rub

WHAT ARE THE RISKS IN DOING THIS EXPERIMENT?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Agar plates may culture dangerous bacteria.	Take care not to open agar plates once they have been incubated. Autoclave used plates for safe disposal.
Liquid soap or alcohol-based hand rub may be irritating to people with sensitive skin.	If you know you cannot use one of these products, inform your teacher or arrange to use the alternative one.

Procedure

1 You will conduct this experiment in pairs. One person will use an alcohol-based hand rub and the other will use soap and water to wash their hands. Form a hypothesis before beginning.
2 Label your agar plates on the underside with your name, the date and your treatment. Label one plate 'before washing' and the other 'after washing'.

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3 Remove the lid from the plate labelled 'before washing' and press the palm of one hand down firmly on the agar, covering as much of the plate as possible. Replace the lid.
4 Following the guidelines, wash your hands using either the alcohol-based hand rub or soap and water.
5 Without touching anything, repeat step 3 using the opposite hand on the plate labelled 'after washing'.
6 Place the plates upside down (agar layer on top), seal with clear tape or Parafilm and incubate at 25°C for 24 hours.

Results

1 Count the number of colonies on each agar plate before and after handwashing. Record your results in a table similar to Table 14.3. Combine all class data to increase sample size.

TABLE 14.3 Results of experiment comparing alcohol-based hand rub with soap and water

PAIR	ALCOHOL-BASED HAND RUB			SOAP AND WATER		
	NUMBER OF COLONIES BEFORE WASHING	NUMBER OF COLONIES AFTER WASHING	PERCENTAGE REDUCTION IN NUMBER OF COLONIES	NUMBER OF COLONIES BEFORE WASHING	NUMBER OF COLONIES AFTER WASHING	PERCENTAGE REDUCTION IN NUMBER OF COLONIES
1						
2						
...						
MEAN						
SD						

Analysis of results

1 Calculate the percentage reduction in number of colonies for each treatment.
2 Calculate the mean percentage reduction in number of colonies for each treatment.

Discussion

1 Compare the mean percentage reductions for the two treatments. Is there any difference? Do your results support your hypothesis?
2 Identify some potential sources of error in this experimental design.
3 Explain why you have calculated the percentage reduction in number of colonies, rather than comparing the number of colonies remaining for each treatment.
4 Do you think that it would be better to use the same hand or the opposite hand for the control plate? Justify your response.
5 In hospitals, it is not just the ability of the treatment to reduce the number of bacteria on hands that influences the transmission of infection. Make a list of other factors that might influence whether alcohol-based hand rubs or soap and water are more effective in reducing nosocomial (hospital) infections.
6 Design an experiment to test these two treatments in the hospital environment. Ensure that you list appropriate control(s) and what outcomes you will measure.

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14.2 INVESTIGATION

Investigating antibiotic resistance

Background

Antibiotics are molecules that are produced by bacteria and fungi as a defence against other microbes. Penicillin was a revolutionary discovery for the human race in the 20th century. Penicillin, along with other antibiotic discoveries, suddenly provided us with a weapon against an invisible enemy. Antibiotics have been harnessed by scientists and medical professionals for treating disease and saving lives. Since that first discovery of antibiotics, they have been developed for use against the broad range of pathogenic microbes. Unfortunately, this weapon has become dulled in the past decade, because overuse has led to antibiotic resistance. Antibiotic resistance occurs when bacteria evolve to become resistant to the antibiotics that have been used to fight them. As a result, antibiotic medicines are not able to kill certain bacteria as effectively, and medical professionals have been forced to find alternative solutions. Not all antibiotics work against all bacteria, and knowing which bacteria are susceptible is essential to finding the best treatment for disease.

Aim

Investigate antibiotic effectiveness against common bacterial strains

Time requirement

45 minutes

Materials

- *Escherichia coli* broth culture
- *Staphylococcus epidermidis* broth culture
- 4 nutrient agar plates
- 2 sterile pipettes
- 2 disposable spreaders
- 2 Mastring antibiotics discs
- Measuring ruler or callipers
- Adhesive tape
- Permanent marker
- Ethanol or bleach
- Bunsen burner
- Forceps
- Contaminated waste bag
- Sterile forceps
- Incubator
- PPE: lab coats, safety glasses, disposable gloves

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
While lab strains are usually harmless, bacteria may cause disease, so assume them to be pathogenic.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end of activity. Decontaminate benches before and after activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Disposable gloves may pose an allergy risk	Use a type of glove with no allergy risk and is suitable for the chemicals being used.

Procedure

1 To use an aseptic technique, wipe your bench down with ethanol (or bleach) and keep your work near the Bunsen burner to take advantage of the updraft the flame will create to waft potential contaminants away from your materials.

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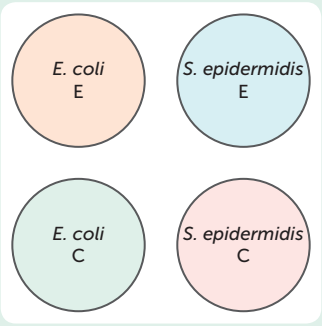
2 Label the bottom of your four agar plates with your name and the date. Label two plates '*E. coli* ' and two plates '*S. epidermidis*'. Label one plate of each type of bacteria with 'E' for experiment and label the other 'C' for control.



3 Using a sterile plastic pipette, transfer a drop of the *E. coli* bacterial broth onto the surface of the agar on your two *E. coli* plates.

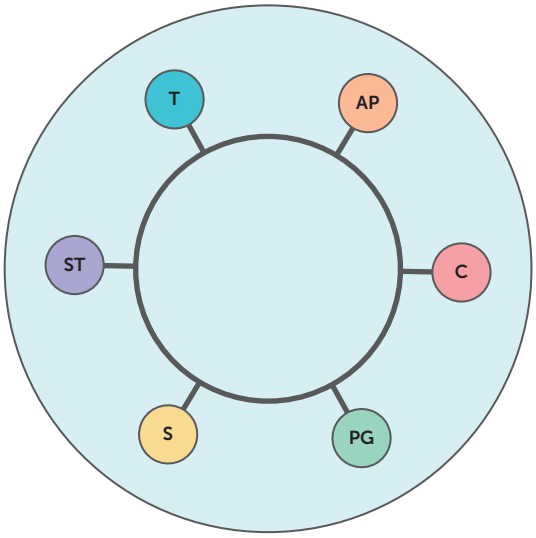
4 Working in close proximity to the Bunsen burner, use the spreader to spread the bacterial broth over the plates evenly. If you are using a glass spreader, pass it through the flame of the Bunsen burner before each use.

5 Replace the lid on the plate immediately to avoid contamination.

6 Repeat steps 3–5 for *S. epidermidis* using a new sterile plastic pipette and spreader.

7 The next step is to apply the Mastring to each of the experiment plates. Wait 10–15 minutes before applying the Mastring to ensure the bacteria has a chance to grow.

8 To apply the Mastring, flame your forceps and allow them time to cool before picking them up. Place the Mastring in the middle of your plate and push (very gently) with the forceps to help it stay in place. Each lobe of the Mastring is impregnated with a different antibiotic; use the code on the packet to differentiate them.



The symbols indicate antibiotics as follows:

AP	Ampicillin (apricot)
S	Streptomycin (yellow)
C	Chloramphenicol (pink)
ST	Sulphatriad (mauve)
PG	Penicillin (green)
T	Tetracycline (blue)

9 Repeat steps 7 and 8 for the other experiment plate, flaming the forceps between each application.

10 Seal all four plates with sticky tape and incubate for 24 hours at 37°C, upside down so that the agar is at the top.

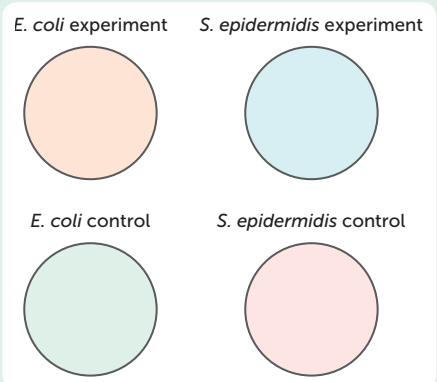
11 Wipe your bench down with ethanol and clean your hands thoroughly.

12 Dispose of all materials safely in a contaminated-waste bag.

13 The next day, observe for the presence or absence of growth near the disc, and measure the diameter of any zones of inhibition. Record your results and contribute to the class data pool.

Results

1 Draw a diagram of what you see on each plate. Include labels.



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2 Copy and complete the table below with the results of your experiment.

ANTIBIOTIC	DIAMETER OF ZONE OF INHIBITION (mm)	
	Escherichia coli	*Staphylococcus epidermidis*
Ampicillin		
Chloramphenicol		
Streptomycin		
Sulphatriad		
Penicillin		
Tetracycline		

3 Calculate the class average diameter of the zone of inhibition for each antibiotic. Copy and complete the table below with the results of your experiment.

ANTIBIOTIC	AVERAGE DIAMETER OF THE ZONE OF INHIBITION (mm)	
	Escherichia coli	*Staphylococcus epidermidis*
Ampicillin		
Chloramphenicol		
Streptomycin		
Sulphatriad		
Penicillin		
Tetracycline		

Discussion

1 Explain the function of the control plate in the experiment. How could a control plate be helpful in the event there was no growth on the experiment plate?
2 What were four variables that you kept constant in this experiment? How did you control them?
3 Why is it important to pool data from the class results to find the average zone of inhibition for each antibiotic?
4 What is a zone of inhibition? How were zones of inhibition created in your experiment?
5 Which antibiotic had the greatest zone of inhibition? Explain why this might be.
6 Did your individual results differ from the class results? If so, suggest possible reasons.
7 Which antibiotic would be most suitable for treating an infection by *Staphylococcus epidermidis*?
8 Which antibiotic would you use if you were unsure of the pathogen in an infection? Explain your answer.
9 Did your results show any signs of antibiotic resistance?
10 Discuss the impacts that antibiotic resistance has on medical treatment?
11 Why have antibiotics become a less effective treatment for infection in recent years?

Taking it further

Test the efficacy of natural antibiotics on similar bacteria.

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CHAPTER 14 SUMMARY

Chapter 14 Activity sheet

- Infectious diseases are spreading more rapidly because of globalisation, travel and trade. Without rapid management by governments and communities, infectious diseases can cause unprecedented harm to people, economics and the environment.
- Management strategies are a coordinated response involving prevention, control and treatment. Specific infectious diseases require specific management plans.
- Quarantine, immunisation, disruption of a pathogen life cycle, medications and physical preventative measures are examples of measures that can be introduced to prevent the spread of infection.
- To predict the spread of disease, mathematical models are used. Mathematicians and biologists work closely to include the complex factors to maximise the reliability of the model. Such models are used to inform public health interventions, such as mass vaccination programs and a safe time to cease self-isolation.
- Immunisation is an important tool in preventing the spread of disease to individuals and throughout a population (through herd immunity).
- Quarantine is an isolation measure used to prevent the spread of disease into healthy populations. In Australia, the focus of quarantine policy has changed over time from human diseases to plant and animal pathogens that threaten our unique wildlife.
- Mathematical models are used to predict the spread of disease as well as to simulate possible outcomes of specific interventions, such as social restrictions. Modelling has improved enormously with supercomputing, which processes higher volumes of data faster while simultaneously performing incredibly complex calculations. Data involving the complexities of disease factors such as population size, environmental change, pathogen persistence and antibiotic resistance can be used as parameters and processed to simulate spread and predict the rate and distribution of new cases emerging.
- Medications such as antibiotics, which inhibit or destroy bacteria, and antivirals, which inhibit the replication of viruses, are effective for some diseases but have limitations due to specificity and drug resistance.
- Handwashing, specialised masks and sneezing into elbows are examples of physical preventative measures used to control the spread of disease.
- Management of an outbreak of a disease, especially an emerging disease, requires an investigation to discover the causative factors.
- Systems for monitoring the spread of disease are needed so that public health interventions can be well targeted. The most common form of disease monitoring involves the reporting of notifiable diseases.

CHAPTER 14 GLOSSARY

Antibiotic An antimicrobial chemical that inhibits or destroys bacteria

Antibody A special protein that is produced by white blood cells and reacts with and helps make pathogens harmless; antibodies are also known as immunoglobulins and are produced by specialist white blood cells called B cells

Antimicrobial agent A medication used to treat infectious diseases

Antiviral An antimicrobial chemical that inhibits the ability of viruses to replicate

Biosecurity A set of strategies that support the prevention of, response to and recovery from diseases that affect our economy, environment and health

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Carrier In reference to infectious diseases, a carrier is an organism that has the infection and is capable of passing it on to others but may or may not show symptoms.

Case An individual who is infected with an infectious disease

Case definition A definition that includes a particular disease, time and place, and is used to help identify individuals affected by a disease outbreak

Contact tracing A process for identifying potential cases; recent contacts of an infected individual are contacted and screened for the infection

Control measure A strategy that reduces the incidence and duration of a disease; it involves meticulous preparation and rapid response to outbreaks at community, state, national and global levels

Definitive host The host in which a pathogen replicates sexually; in the case of malaria, sexual reproduction of *Plasmodium* occurs in the gut of the female mosquito

Emerging disease A disease that has recently appeared in a population, has occurred previously but affected only small numbers in isolated places, or has occurred previously but only recently been recognised as being due to a newly identified pathogen

Endemic A disease that is always present in a population or region

Epidemic An increase in the occurrence of a specific disease above the baseline level for a particular population; it tends to refer to larger, more serious events than an outbreak

Epidemiologist A scientist who studies the causes and effects of diseases at a population level, and who works to prevent or minimise the impact of diseases on the population

Epidemiology The study of the occurrence of disease in populations

Fomite A surface or non-living object carrying an infectious agent

Herd immunity The phenomenon that once a particular proportion of a population is immune to a disease, susceptible individuals are also better protected from the specific disease

Host An organism that is infected by a pathogen

Immunisation The act of protecting someone from disease by the use of a vaccine; the process of developing resistance to a specific disease

Index case The patient zero (initial patient) in the population of an epidemiological investigation

Infectivity The ability of a pathogen to spread from one host to another potential host

Intermediate host The host in which a pathogen replicates asexually; in malaria, this occurs in the liver and red blood cells of a human

Management strategy A coordinated response to an infectious disease involving prevention, control and treatment; specific infectious diseases require specific management plans

Mortality The impact of a disease within a population, measured by the number of deaths caused by that disease

Notifiable disease A disease that, if diagnosed, is required to be reported to public health authorities

Outbreak A sudden, unexpected increase in the prevalence of a particular disease above the baseline level for that population; it could be a single case of a contagious disease in a small community

Pandemic A disease that has spread rapidly throughout the world; an epidemic that has crossed international borders

Patient zero The index case (initial patient) in the population of an epidemiological investigation

Persistence Refers to the ability of a pathogen to survive for long periods of time in reservoirs, or to how long the pathogen remains viable outside the host

Prevention Preventing transmission of a disease, onset of disease signs and symptoms, and impact on the environment or society

Quarantine A period of isolation serving to prevent the spread of a contagious disease; suspected cases are isolated from local, susceptible populations until at least the incubation period is finished, clinical signs and symptoms have passed and a scientist confirms the suspected pathogen is no longer present

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Replication In reference to diseases, the process of producing new pathogens from old pathogens

Sign An objective and measurable experience of a pathogen host that is directly observable: elevated body temperature, breathing rate, pulse rate and/or blood pressure are important 'vital' signs of disease

Symptom A subjective experience felt by a patient, such as nausea or pain

Treatment Health provisions such as medication and vaccination that treat or prevent disease

Vaccination The administration of a vaccine to the bloodstream to cause immunity, usually by injection; immunisation is what happens in your body after you have had the vaccination

Vaccine A treatment containing a dead or weakened/inactive form of a pathogen that stimulates a specific immune response; vaccines contain antigen components harvested from an infected organism and stimulate the production of antibodies

Vector In reference to diseases, an agent that transmits pathogens from one host to another; in genetics, a vehicle used to transfer DNA sequences from one organism to another

Virulence The ability of a pathogen to cause severe disease within its host

CHAPTER 14 REVIEW QUESTIONS

Remembering

1 List five general management strategies used to control the spread of disease.
2 State the role of an epidemiologist in managing the spread of disease.
3 Describe the impact of increased travel and trade on disease transmission in the context of:
   a island-based wildlife
   b emerging human viruses.
4 Using an example, describe herd immunity.
5 Outline how hand hygiene can help prevent the transmission of disease.

Understanding

6 Explain why herd immunity cannot be an effective management strategy against tetanus.
7 Some people are unable to receive vaccinations because of a medical condition. Explain why the vaccination of healthy individuals is important for those who cannot receive vaccinations.
8 Explain why it is important to collect data about disease rates, even when levels are fairly stable.
9 Research a recent outbreak of an emerging/re-emerging disease (such as SARS or swine fever) and describe the management strategies used to control the spread. Include what was done and who did it (WHO, UN, government or community).

Applying

10 TB is caused by a bacterium that can lie latent in the body for many years. When it reactivates, it can cause serious infections of any part of the body, but most commonly the lung. When diagnosed, patients with reactivated TB are treated for 2 weeks in hospital isolation before continuing treatment at home. All visitors must wear masks to enter the isolation unit.
   a Explain why patients are initially treated in isolation.
   b Is this a type of quarantine? Explain why or why not.
   c Public health authorities will contact and test other people in the household of somebody diagnosed with TB. Name this process.

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11 Malaria is a disease that is a significant cause of mortality worldwide.

a Summarise the life cycle of the malaria parasite, *Plasmodium*.

b Explain how this life cycle affects the distribution of this disease.

There are several conditions caused by abnormal alleles of the gene that codes for haemoglobin. One of these abnormal alleles, the sickle cell allele (*h*), causes red blood cells produced to have abnormal shapes. For patients who are homozygous (*hh*) for this allele, these abnormally shaped red blood cells cause a serious disease known as sickle cell anaemia. However, heterozygotes, who also have one normal allele (*Hh*), are usually unaffected. Heterozygotes are also more resistant to infection with malaria than are people with two normal alleles (*HH*).

c In areas where malaria is common, which genotype would provide an individual with the biggest selective advantage: *HH*, *Hh* or *hh*? Justify your response.

d Sickle cell anaemia is much more common in parts of the world near the equator. Linking this to your knowledge of evolution, explain why this is the case.

12 Influenza is a viral infection that is most common in winter. It is spread from person to person by airborne droplets produced when sneezing or coughing. The rapid evolution of the virus means that individuals who have been exposed to one strain may not have immunity against other strains.

a Suggest some disease, population and environmental characteristics that may explain why influenza is most common in winter.

b Explain how handwashing can be used to prevent the spread of influenza.

c Explain why herd immunity is not able to provide complete protection against influenza.

Analysing

13 Draw a table that compares disease surveillance and predictive modelling (including the type of information and when each technique is useful).

Evaluating

14 Evaluate the current management strategies used to control Ross River virus disease.

Creating

15 Create a Venn diagram to summarise the management strategies used in controlling the spread of phytophthora and crown gall diseases.

PRACTICE EXAM QUESTIONS

1 A pandemic is most likely to arise from a new influenza virus strain that

A spreads easily among humans

B causes a high mortality rate in humans

C cannot replicate in humans

D has the same protein coat as an existing strain.

[Q16 2018 SCSA]

2 The following is a list of the main steps in the life cycle of a virus, listed in no particular order.

Step A Viral proteins and nucleic acids are assembled in the host cell.

Step B The virus binds to the host cell.

Step C The virus injects its nucleic acid into the host cell.

Step D The host cell releases viral particles.

Step E The host cell produces viral nucleic acids and proteins.

Which of the following lists these steps in the order in which they occur in the life cycle of the virus?

A B → C → E → A → D

B A → E→ C → D → B

C C → B → A → D → E

D D → C → E → B → A

[Q23 2017 SCSA]

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3 Tuberculosis will spread most rapidly through a host population when the density of the host population is

A high and herd immunity is low.
B high and herd immunity is high.
C low and herd immunity is low.
D low and herd immunity is high.

[Q2 2016 SCSA]

4 Select a true statement about TB.

A TB cannot be prevented.
B People with latent TB can transmit the disease.
C TB can usually be treated with a 6-month course of antimicrobial drugs.
D Multidrug-resistant tuberculosis is caused by bacteria that does not respond to the standard course of medication and requires a course of antiviral drugs.

[Q8 2016 SCSA]

5 Outline two distinctly different methods of controlling the spread of malaria. (4 marks)

[Q35c 2018 SCSA]

6 Discuss management strategies that can be used to control the spread of phytophthora dieback disease. (6 marks)

[Q39 2018 SCSA]

7 A group of biologists developed a model for predicting the spread of influenza in human populations. As a part of this, they collected data on the number of individuals per household in two locations, shown in Figure 14.19.

Compare the number of people per household in the two locations. Use data from Figure 14.19 to support your answer. (4 marks)

[Q32a 2017 SCSA]

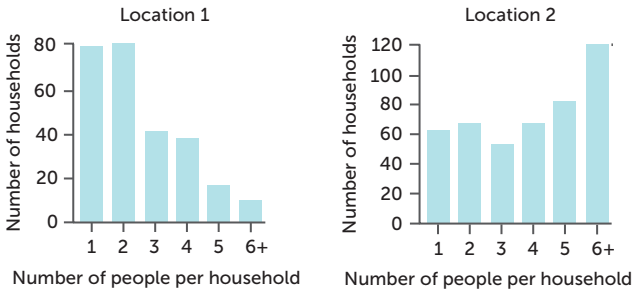

FIGURE 14.19 Number of people per household

8 Explain why data on the number of people per household are relevant to the development of a model for predicting the spread of influenza in human populations. (4 marks)

[Q32b 2017 SCSA]

9 Can influenza be treated with antibiotics? Explain why or why not. (4 marks)

[Q32c 2017 SCSA]

10 White spot disease is a highly contagious viral disease that affects prawns. It is found in parts of Asia, America, Africa and the Middle East, but generally not in Australia. A recent outbreak has, however, occurred in several prawn farms on a river in Queensland. Explain two measures that could be taken to reduce the risk of white spot spreading from the affected farms to other parts of Australia. (4 marks)

[Q32e 2017 SCSA]

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